World Journal of Gastroenterology®

Volume 12 Number 34
September 14, 2006

Supported by NSFC
2005-2006

National Journal Award
2005

The WJG Press

The WJG Press, Apartment 1066 Yishou Garden, 58 North Langxinzhuang Road, PO Box 2345, Beijing 100023, China
Telephone: +86-10-85381901
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http: //www.wjgnet.com

ISSN 1007-9327 CN 14-1219/R Local Post Offices Code No. 82-261

World Journal of Gastroenterology
www.wjgnet.com
Volume 12
Number 34
Sep 14
2006

ISSN 1007-9327
CN 14-1219/R

World Journal of Gastroenterology®

Indexed and Abstracted in:
Current Contents/Clinical Medicine and Science Citation Index-Expanded, Index Medicus, MEDLINE and PubMed, Chemical Abstracts, EMBASE/Excerpta Medica, Abstracts Journals, Nature Clinical Practice Gastroenterology and Hepatology, CAB Abstracts and Global Health.
ISI JCR 2003-2000 IF: 3.318, 2.532, 1.445 and 0.993.

Volume 12 Number 34
September 14, 2006

World J Gastroenterol
2006 September 14; 12(34): 5429-5592
Online Submissions
www.wjgnet.com/wjg/index.jsp
www.wjgnet.com
Printed on Acid-free Paper

A Weekly Journal of Gastroenterology and Hepatology

National Journal Award
2005

World Journal of Gastroenterology®

Volume 12 Number 34
September 14, 2006

Supported by NSFC
2005-2006

Contents

World Journal of Gastroenterology
Volume 12 **Number** 34 **September** 14, 2006

Contents

World Journal of Gastroenterology
Volume 12 **Number** 34 **September** 14, 2006

Contents

COPY EDITOR FOR THIS ISSUE: Dr. SD Taylor-Robinson

World Journal of Gastroenterology (*World J Gastroenterol , WJG*), a leading international journal in gastroenterology and hepatology, has an established reputation for publishing first class research on esophageal cancer, gastric cancer, liver cancer, viral hepatitis, colorectal cancer, and *H pylori* infection, providing a forum for both clinicians and scientists, and has been indexed and abstracted in Current Contents/Clinical Medicine and Science Citation Index-Expanded, *Index Medicus*, MEDLINE and PubMed, Chemical Abstracts, EMBASE/Excerpta Medica, Abstracts Journals, Nature Clinical Practice Gastroenterology and Hepatology, CAB Abstracts and Global Health. ISI JCR 2003-2000 IF: 3.318, 2.532, 1.445 and 0.993. *WJG* is a weekly journal published by The *WJG* Press. The publication date is on 7th, 14th, 21st, and 28th every month. The *WJG* is supported by The National Natural Science Foundation of China, No. 30224801 and No.30424812, which was founded with a name of *China National Journal of New Gastroenterology* on October 1,1995, and renamed as *WJG* on January 25, 1998.

PUBLISHED BY
The WJG Press

PRINTED BY
Printed in Beijing on acid-free paper by Beijing Kexin Printing House

 Print ISSN 1007-9327
CN 14-1219/R.

EDITORIAL OFFICE
World Journal of Gastroenterology,
The WJG Press, Apartment 1066 Yishou Garden, 58 North Langxinzhuang Road, PO Box 2345, Beijing 100023, China
Telephone: +86-10-85381901
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

SUBSCRIPTION AND AUTHOR REPRINTS
Jing Wang
The WJG Press, Apartment 1066 Yishou Garden, 58 North Langxinzhuang Road, PO Box 2345, Beijing 100023, China
Telephone: +86-10-85381901
Fax: +86-10-85381893
E-mail: j.wang@wjgnet.com
http://www.wjgnet.com

Institutional Rates
2006 rates: USD 1500.00

Personal Rates
2006 rates: USD 700.00

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/wjg/help/instructions.jsp. If you do not have web access please contact the editorial office.

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5429-5439
World Journal of Gastroenterology ISSN 1007-9327

EDITORIAL

Human hepatic sinusoidal endothelial cells can be distinguished by expression of phenotypic markers related to their specialised functions *in vivo*

PF Lalor, WK Lai, SM Curbishley, S Shetty, DH Adams

PF Lalor, WK Lai, SM Curbishley, S Shetty, DH Adams, Liver Research Group, Institute of Biomedical Research, Division of Medical Science, University of Birmingham, Birmingham B15 2TT, United Kingdom
Correspondence to: Dr. Patricia Lalor, Liver Research Group, Institute of Biomedical Research, Division of Medical Science, University of Birmingham, Birmingham B15 2TT, United Kingdom. p.f.lalor@bham.ac.uk
Telephone: +44-121-4158784 Fax: +44-121-4158701
Received: 2006-05-08 Accepted: 2006-06-14

Abstract

The hepatic sinusoids are lined by a unique population of hepatic sinusoidal endothelial cells (HSEC), which is one of the first hepatic cell populations to come into contact with blood components. However, HSEC are not simply barrier cells that restrict the access of blood-borne compounds to the parenchyma. They are functionally specialised endothelial cells that have complex roles, including not only receptor-mediated clearance of endotoxin, bacteria and other compounds, but also the regulation of inflammation, leukocyte recruitment and host immune responses to pathogens. Thus understanding the differentiation and function of HSEC is critical for the elucidation of liver biology and pathophysiology. This article reviews methods for isolating and studying human hepatic endothelial cell populations using *in vitro* models. We also discuss the expression and functions of phenotypic markers, such as the presence of fenestrations and expression of VAP-1, Stabilin-1, L-SIGN, which can be used to identify sinusoidal endothelium and to permit discrimination from vascular and lymphatic endothelial cells.

Key words: Human; Liver; Endothelium; Sinusoid; Phenotype

Lalor PF, Lai WK, Curbishley SM, Shetty S, Adams DH. Human hepatic sinusoidal endothelial cells can be distinguished by expression of phenotypic markers related to their specialised functions *in vivo*. *World J Gastroenterol* 2006; 12(34): 5429-5439

http://www.wjgnet.com/1007-9327/12/5429.asp

INTRODUCTION

The liver has a unique dual blood supply receiving blood from both the portal vein and the hepatic artery. Unlike other organs, which are supplied by arterial blood through arterioles, the liver receives venous blood at low pressures through the portal vein as well as arterial blood *via* the hepatic artery. The intrahepatic portal venous system consists of conducting and distributing systems that ensure blood is carried throughout the parenchyma and evenly delivered to individual hepatocytes *via* the sinusoidal network[1]. A uniform and strict branching pattern appears at the level of the terminal vessels and is maintained throughout the distributing system. The first order branches of the distributing system arise from the terminal branches of the conducting system and give rise to second order vessels of approximately 70 μm diameter that correspond to the terminal portal vein branches seen in portal tracts. Third order vessels arise from these and correspond to the septal or interlobular branches. These exhibit a classical sinusoidal appearance, lack a connective tissue sheath and have no basement membrane. The distributing system then branches into the hexagonal lobule before draining into the hepatic vein. The hepatic artery supplies 25% of hepatic blood flow and provides blood to both the parenchyma and the portal tracts. Axial hepatic arterial branches run parallel to the conducting portal veins and terminate in inlet venules, terminal portal veins and the sinusoids, thereby supplying blood to the parenchyma. The axial arteries also give rise to peribiliary branches that supply the accompanying bile ducts and portal interstitium. These arteries form the peribiliary plexus consisting of efferent and afferent capillaries that wrap around the surface of bile ducts. Small channels from the peribiliary plexus drain into the sinusoids or portal vein branches. Thus, complex anastomoses exist between the axial arteries and peribiliary arteries[2].

ISOLATION AND CULTURE OF HEPATIC SINUSOIDAL ENDOTHELIAL CELLS

Isolated cultures of hepatic sinusoidal endothelial cells present a valuable tool for the study of liver physiology and pathophysiology. Most published studies used cells isolated from rodent livers and have defined the role of fenestrations and specific receptors in uptake and

processing of circulating factors including pathogens (reviewed in[3]). Most investigators use a combination of enzymatic digestion and density gradient centrifugation to isolate HSEC from liver tissue although there is considerable variation in the protocols used by different groups and for cells from different species. HSEC from rodents are commonly isolated by enzymatic digestion, either by perfusion of an intact organ with an enzymatic cocktail or by mechanical disruption followed by enzymatic digestion[4-7]. The cell suspension generated by such methods is fractionated using differential centrifugation techniques, including counterflow elutriation or density gradient centrifugation. Endothelial cells are then grown on matrix coated flasks in selective growth media containing growth factors.

Similar methods can be used to isolate HSEC from human liver samples[8-10] and between 10^3-10^6 cells have been isolated from whole livers unsuitable for transplantation[9]. However, the limited access to intact human liver means that most groups use diseased tissue removed at transplantation or surgical resection or biopsy specimens that generate low numbers of cells per isolation. Nevertheless, successful isolation is aided by the large numbers of HSEC relative to other vascular endothelial cells present in the liver. Some researchers further reduce the potential of contaminating vascular endothelium by excising visible large vascular structures from the liver tissue prior to enzymatic digestion. Although several different isolation procedures have been described these are not all equally effective in our hands. Our experience, and that of other groups, suggests that counterflow elutriation alone is not useful for selection of human HSEC from a mixed non-parenchymal cell preparation as most of the cells have similar centrifugal densities[6,11]. This led us to routinely include a step of immunomagnetic depletion to remove common contaminating cell populations, such as biliary epithelial cells, followed by positive selection of endothelial cells using antibody against CD31 (see later)[10]. Similar methods using magnetic beads coated with Evonymus europaeus agglutinin have been used to isolate HSEC from the liver of other primates[12] and there are now commercial antibody-based magnetic kits for isolation of rodent HSEC. Despite these refinements there is considerable variability in the yield and viability of cells obtained from diseased human liver tissue. Thus, although there are compelling reasons to study human HSEC *in vitro* low cell yields mean that it is difficult to use primary cells without passage.

PROBLEMS WITH EXISTING PHENOTYPIC MARKERS AND USE OF HSEC IN MONOCULTURE

As scientific technology advances it is possible to carry out increasingly complex genetic, proteomic and functional analyses on cells grown in culture. However such techniques require highly purified populations of cells of defined phenotype. Some of the methods previously used to confirm HSEC purity and phenotype, such as AcLDL uptake[8] and binding of Ulex lectin[8], are not specific. For example, other hepatic cell populations, including dendritic cells, take up AcLDL and Ulex lectin binds to fucosylated receptors on both Kupffer cells and HSEC[13]. Similarly, endothelial cells share many cell surface receptors with leukocytes, including CD31, CD4[14], CD11b, and CD11c[11], which may contaminate endothelial cell preparations in culture. Care also needs to be taken when using antibody staining to define HSEC phenotype because HSEC express high levels of FcγR[15] allowing them to bind antibodies non-specifically. In light of such problems, it has been suggested that the presence of open fenestrations arranged in sieve plates is the only true marker of hepatic sinusoidal endothelial cells[16]. These pores are indeed classic features of liver sinusoidal endothelial cells *in vivo* but present problems when used to identify cells *in vitro* (see below).

All of these problems are compounded by the fact that HSEC are most commonly cultured as a monolayer of cells on matrix-coated tissue culture plates *in vitro*. This perturbs the normal morphology of the cells and they become flattened and rapidly lose fenestrations. Part of this effect may be the loss of paracrine signals from other cells of the sinusoid that maintain the phenotype and differentiation of HSEC *in vivo*. For example, crosstalk between hepatic sinusoidal endothelial cells and closely juxtaposed hepatocytes is essential for the maintenance of sinusoidal endothelial cell growth and differentiation. This is demonstrated by studies where implantation of foetal liver fragments into quail chorioallantoid membrane resulted in the acquisition of a sinusoidal phenotype by the chorioallantoid microvessels[17] and also *in vitro* studies where co-culture of HSEC with other liver cells resulted in a more stable endothelial phenotype and function[18]. Thus, markers used to determine phenotypes of HSEC must take into account alterations in phenotype as a consequence of culturing cells in isolation in the absence of local paracrine signals.

FENESTRATIONS

Endothelial cells throughout the adult organism are derived from common early embryological precursors and have broadly similar functions and histological appearance. However, there is important organ and tissue-specific heterogeneity that results in phenotypic and functional variations (reviewed in[19]). For example, high endothelial venules in lymph nodes are lined by morphologically and phenotypically distinct endothelial cells that have the unique ability to promote the recruitment of naïve lymphocytes whereas lymphatic endothelium express several receptors that allow uptake of macromolecules found in lymph[20]. Sinusoidal endothelial cells are found in the spleen and bone marrow, as well as in the liver, and in all these sites they have a minimal basement membrane and lack classical tight junctions. Hepatic sinusoidal endothelium differs from sinusoidal endothelium in these other beds by its discontinuous nature, being interspersed with kupffer cells and by the presence of open fenestrations arranged in sieve plates[16].

The vascular architecture in the human liver develops by 17-25 wk of gestation, and the sinusoids acquire their

Table 1 The expression of classical markers of endothelial phenotype by human sinusoidal endothelial cells

Marker	Extra-hepatic endothelial expression	Sinusoidal endothelial cells *in vivo*	Sinusoidal endothelial cells *in vitro*	Problems with use in phenotyping?
CD31	Vascular and lymphatic endothelial cells	Yes, but at low levels	Yes	Widely expressed on all EC thus not specific for HSEC
vWF	Vascular endothelial cells	Controversial, Yes	Yes	Widely expressed on all EC thus not specific for HSEC
Ulex lectin binding	Endothelial cells	Yes	Yes	Widely expressed on all EC thus not specific for HSEC
Uptake of AcLDL	Endothelial cells	Yes	Yes	Also taken up by macrophages in liver and other EC
CD34	Vascular and lymphatic endothelial cells	No	Absent or low	May be upregulated during capillarisation or with passage *in vitro*
E-Selectin	Vascular endothelial cells	Low or absent under normal conditions	Low, can be upregulated by cytokines	Widely expressed on activated vascular EC, not specific for HSEC
Pal-E antigen	Vascular endothelial cells	No	No?	May be upregulated during capillarisation
CD105/ endoglin	May be upregulated during capillarisation	Yes	Yes	Widely expressed on all EC, also by stellate cells and fibroblasts in liver

While many of the above markers are indeed expressed on hepatic sinusoidal endothelial cells and provide a means of confirming "sinusoidal endothelial identity", none is specific to sinusoidal endothelial cells.

distinctive fenestrated phenotype by wk 20 (reviewed in[21]). The fenestrations act as a 'dynamic filter'[3] allowing macromolecules in blood controlled access to parenchymal cells[22]. Evidence from animal studies suggests that fenestrations constitute up to 40% of the cell and that the size, distribution and clustering of the pores in sieve plates varies with the zonal distribution of the endothelium[23] and across the endothelial surface. Although normal hepatic sinusoidal cells in most mammals are characterised by the presence of fenestrations (reviewed in[3]), caution must be exercised when translating these observations to human cells. Studies of fenestrations in human liver samples are rare, and suggest that the number and size of fenestrations differs from that observed in other mammals[3]. Furthermore, the number of fenestrations per endothelial cell decreases in disease[24,25], following viral infection[22] or with ageing[26]. During cirrhosis and chronic hepatitis, HSEC develop a more vascular morphology and produce a basement membrane in a process known as 'capillarisation' (reviewed in[27]). This is associated with increased expression of CD31 and VCAM-1 and loss of fenestrations[27]. These changes may impede the transfer of materials to and from the parenchyma and contribute towards regional hepatocyte hypoxia. Fenestrations are not unique to hepatic EC but are found in endothelium in endocrine glands, kidney, gastrointestinal tract, choroid plexus, lymphatic organs such as the spleen and are sometimes observed in tumour vasculature. Many studies have implicated VEGF as an essential factor for regulation of fenestrations in these organs (reviewed in[28]).

Thus considerable variations in the number, size and localisation of fenestrations are seen among species and also in health and disease. The situation becomes more complex when cells are removed from the hepatic microenvironment and cultured *in vitro*. The fenestrations documented in freshly isolated rat HSEC begin to disappear within 48 h of cell culture[29] and are almost gone within a week[30]. We have made similar observations with human cells from normal livers and also find very few fenestrated cells when HSEC are isolated from cirrhotic livers (Lalor and Adams unpublished observations). However, the number of fenestrations on rat HSEC can be maintained *in vitro* by the addition of VEGF and by culturing cells on extracellular matrix constituents, such as collagen, that are secreted by endothelial cells[29,31]. Both human and rodent HSEC need growth factors and attachment to appropriate extracellular matrix molecules to survive and will rapidly undergo apoptosis in the absence of these. Thus in order to maintain cell survival, cultured HSEC must be grown in the presence of VEGF which induces and maintains expression of fenestrations as well as promoting HSEC proliferation. VEGF is also a growth factor for vascular endothelial cells which induces the production of matrix molecules essential for survival and proliferation[32]. At higher concentrations, VEGF can induce the formation of fenestrations in vascular endothelial cells[28,32,33] and, although these pores are not organised into sieve plates, they can be very difficult to distinguish from the fenestrations that characterise HSEC. This inducibility of fenestrations in vascular endothelial cells together with the impracticality of using electron microscopy for routine phenotyping means that the presence of fenestrations alone cannot be used to define HSEC in most experimental situations.

NORMAL 'ENDOTHELIAL' CELL PHENOTYPE AND FUNCTIONS

HSEC form a single cell barrier between the hepatocytes and the bloodstream and are strategically situated to interact with leukocytes and other blood constituents. The cells produce a minimal basement membrane[34], which is mostly composed of type IV collagen in normal liver[35], and have a high endocytotic capacity[3]. They express some markers that are common to all endothelial cells and these provide a useful means to positively identify a cell of 'endothelial' lineage (Table 1).

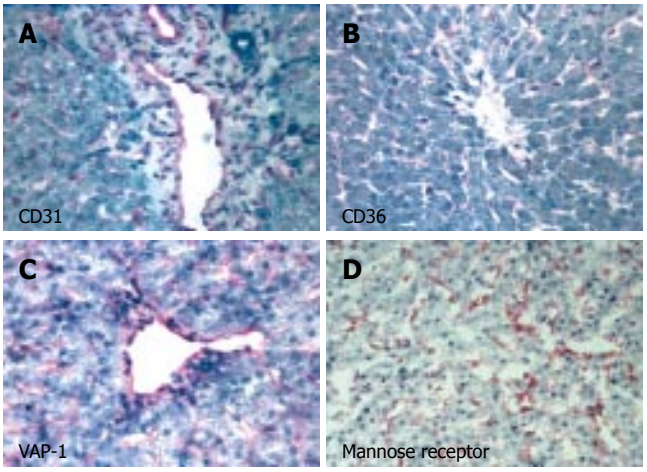


Figure 1 Human hepatic sinusoidal endothelial cells *in situ* express the classical endothelial phenotypic markers CD31 (**A**) and CD36 (**B**) as well as more recently identified markers such as VAP-1 (**C**) and Mannose receptor (**D**). Images represent immunohistochemical staining of human liver sections using specific primary antibodies in an indirect immunoperoxidase protocol with haematoxylin counterstain. Positive staining of sinusoidal endothelial cells is indicated by pink pigmentation. CD31, CD36 and VAP-1 are observed on both portal vessel endothelial cells and sinusoidal endothelium, whilst the mannose receptor is localised on sinusoidal endothelial cells and Kupffer cells.

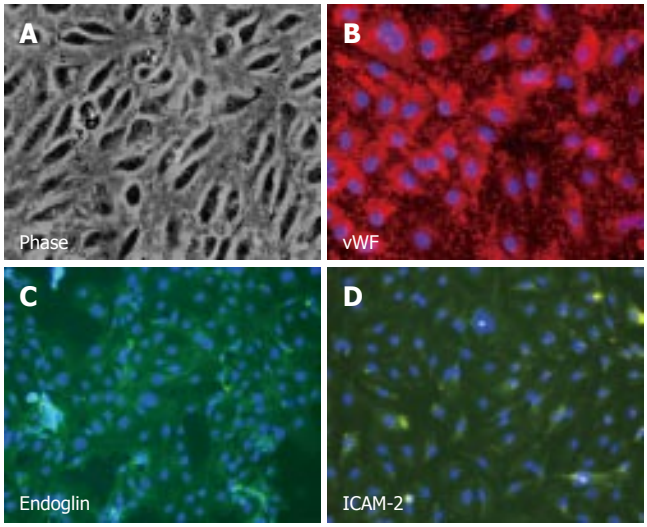


Figure 2 Isolated, cultured human hepatic sinusoidal endothelial cells exhibit classical morphology under phase contrast microscopy (**A**), and stain positively with antibody directed against endothelial phenotypic markers. Images represent immunofluorescent staining of cultured cells using specific primary antibodies in an indirect fluorescent protocol with DAPI nuclear counterstain (blue). Positive staining for vWF (**B**) is visualised using a Texas Red-labelled secondary antibody, whilst expression of endoglin (CD105, **C**) and ICAM-2 (**D**) are visualised with a FITC-conjugated secondary antibody (green).

CD31

CD31 or PECAM-1 is an abundantly expressed membrane glycoprotein member of the immunoglobulin superfamily (reviewed in[36]). It is constitutively expressed on endothelial cells and some haematopoetic cells and has functions in cell adhesion and signalling. Expression of CD31 is used widely as a marker of 'continuous' or classical vascular endothelium, but expression of CD31 by hepatic sinusoidal endothelial cells remains controversial. In rodent studies, CD31 has been localised to normal sinusoidal endothelial cells using a variety of techniques[4,37] and has been shown to be downregulated following CCl_4 or TNFα mediated liver injury[37]. However, other studies suggest that the protein is absent under normal conditions[34] and indeed a lack of CD31 has been used to characterise rodent HSEC[7].

The situation is similarly complex in human HSEC. During human embryonic development CD31 is absent from HSEC until wk 25. In the adult, CD31 has been reported as present on HSEC by FACS and immunohistochemistry on sections from cirrhotic liver but is minimally present on normal liver[8] or alternately present on normal liver and enhanced on cirrhotic liver[38]. Our own data show that CD31 is expressed by both normal and diseased liver HSEC (Figures 1 and 2). In general, it seems that HSEC express lower levels of CD31 than vascular EC ([16] and our observations). However, one needs to interpret immunohistochemical analysis with caution because CD31 is present on kupffer cells in sinusoids. Studies on isolated HSEC using both PCR and antibody-based assessment of characteristic endothelial markers, such as CD31 and vWF (see below) in parallel, show varying results[39]. Some studies suggest that subcellular localisation of CD31 can be used as a marker of HSEC phenotype to indicate whether cells have dedifferentiated in culture[16]. Thus cells with cytoplasmic CD31 are 'normal' whereas dedifferentiated/capillarised EC demonstrate increased membranous expression.

Von Willebrand Factor (vWF)

vWF is a multimeric glycoprotein that binds and stabilises the coagulation factor FVIII as well as supports the adhesion of platelets to subendothelial structures during vascular damage. It is expressed by both platelets and endothelial cells and is often used as a marker to identify endothelium. In most vascular endothelial cells, von Willebrand Factor is stored in cytoplasmic vesicles called Weibel Palade bodies. Expression of vWF varies between different vascular beds *in vivo*[40] and particularly low levels are observed in the liver, most of which is detected in vascular rather than sinusoidal endothelial cells. The low levels of vWF detected in HSEC are consistent with the reported lack of Weibel Palade bodies. However, definitive evidence supporting the presence or absence of these structures is also lacking. Studies in mice suggest that HSEC contain Weibel-Palade bodies[41] and produce VWF at the mRNA and protein level[4,11,41]. Other groups working with rat HSEC report that vWF is not expressed in normal rat cells[42] and these findings are supported by porcine and rat studies showing absence of Weibel-Palade bodies in HSEC[9,43]. In human cells, vWF expression has been reported in both normal[8,44] and diseased samples[45] and we and others[8] have demonstrated expression on passaged, cultured HSEC *in vitro* (Figure 2).

E-Selectin

E-Selectin is a member of the selectin family of adhesion molecules that supports leukocyte binding. Expression of E-Selectin is restricted to cells of endothelial lineage[46]

and is induced by inflammation *in vivo* and exposure of endothelial cells to proinflammatory cytokines and LPS *in vitro*. The ability to express E-selectin can thus be used to define endothelial cells in culture. However, expression of E-selectin is restricted to vascular endothelial cells in the normal liver[47] , although it may be upregulated on sinusoidal endothelium in disease and during metastatic processes[48] and animal studies demonstrate a minimal role for E-selectin in leukocyte recruitment to liver tissue[49]. However, we have reported expression of functional E-Selectin on cultured cytokine-stimulated human HSEC suggesting that HSEC can express E-Selectin under restricted circumstances *in vivo* and *in vitro*[18]. Recent studies demonstrating that E-Selectin expression by HUVEC in response to TNFα is reduced by pretreatment with HGF suggest that paracrine factors from adjacent hepatocytes may suppress E-selectin in the sinusoids *in vivo*[50].

Binding of lectins and Acetylated LDL uptake

The ability to bind Ulex lectin and take up acetylated LDL is often used to define HSEC. Ulex lectin, from the gorse family of plants, binds alpha-L fucose containing receptors and is commonly used as a histological marker for endothelial cells, although in some tissues it also binds epithelial structures[51]. In the liver, different lectins bind differentially within the vasculature. Concanavalin A binds with equal affinity for all segments of the microvasculature whereas wheat germ agglutinins show preferential binding to the sinusoidal vasculature as a consequence of differences in distribution of glycosylated ligands throughout the acinus. In most studies Ulex lectins do not bind preferentially to sinusoidal endothelium as a consequence of the relative paucity of alpha-L fucose motifs. The ability of wheat germ lectins to bind sinusoidal endothelium has led to their use in selectively purifying sinusoidal EC and their preference for periportal HSEC has even led to the suggestion that they can be used to differentially purify periportal verses perivenous HSEC[5]. Staining with Ulex lectin is increased in disease and is particularly pronounced during capillarisation of the sinusoids[52]. Thus although binding of Ulex lectin is indicative of an endothelial phenotype, it is not restricted to 'sinusoidal' endothelial cells[52] and is not a good marker of HSEC.

The liver is the major site for the scavenger receptor-mediated clearance of lipoproteins from the circulation. Acetylated LDL is mainly cleared by hepatic endothelial cells[53] by binding to scavenger receptors including scavenger receptor class A (SR-AI/Ⅱ). However, these receptors are expressed by both HSEC and macrophages[54] and the ability to take up acetylated LDL is common to many extrahepatic endothelial cell populations, again reducing the specificity of acLDL uptake as a characteristic property of HSEC.

CD34

CD34 is a type 1 transmembrane sialomucin expressed by haematopoetic stem cells, capillary and lymphatic endothelial cells. CD34 is absent from most sinusoidal endothelial cells in normal liver but expression increases[8,27,55] during capillarisation in chronic inflammatory disease and in the sinusoidal-type vasculature within hepatocellular carcinomas[56,57]. CD34 expression has also been shown to increase in other tissues including the rheumatoid joint and at sites of neolymphoid development during chronic inflammation. We have described CD34 positive lymphatic-like vessels in portal associated lymphatic tissue in chronic inflammatory liver diseases including PSC[58] and hepatitis C (Heydtmann 2006 *J Immunol* in press). However, CD34 is not expressed on most HSEC in non-inflamed tissue *in vivo* and is absent from low passage human HSEC *in vitro*.

Pal-E Antigen

The antigen recognised by the Pal-E antibody is a widely used marker of vascular endothelial cells[59-61] The identity of the protein has been proposed as either a secreted form of vimentin produced by endothelial cells[61] or a protein designated PV-1/FELS (plasmalemmal vesicle-1/fenestrated endothelial-linked structural protein)[60]. PV-1 is particularly interesting in the context of HSEC. As its name implies, PV-1/FELS is expressed by fenestrated endothelial cells in the kidney and pancreas[62] but it is absent from fenestrated hepatic sinusoidal endothelial cells[60,62] and is restricted to vascular endothelium and neovessels in areas of capilliarisation in chronic liver disease and hepatocellular carcinoma[52,58].

Vascular Endothelial-Cadherin (VE-Cadherin)

Cadherins are a family of adhesion molecules that demonstrate cation- dependent homophilic and heterophilic binding. Endothelial cells express at least three cadherins: N-, P-, and VE-cadherin. VE-cadherin is localised to the inter-endothelial cell junction where it is an essential part of the adherens junctions that maintains endothelial permeability, monolayer integrity, morphogenesis and angiogenic reponses. Most studies suggest that sinusoidal endothelium in normal liver lacks VE-cadherin or expresses it at low levels, although it can be detected in chronic inflammation[38,63]. It seems likely that the relative lack of VE-cadherin on HSEC is a consequence of the absence of classical adherens junctions between HSEC and this is consistent with a lack of other junctional proteins, including vascular endothelial junctional adhesion molecule (VE-JAM/JAM-2), which is a member of the immunoglobulin superfamily structurally similar to JAM-1, which is absent from foetal and adult liver[64].

CD105/endoglin

CD105 (endoglin) is a hypoxia-inducible protein that is widely expressed on endothelial cells and is upregulated during angiogenesis. It is a receptor for transforming growth factor (TGF) -β1 and -β3 and modulates TGF-β signalling by interacting with TGF-β receptors I and/or Ⅱ (for review see[65]). CD105 has been used as a marker of angiogenesis, particularly in tumour tissue[66], and because it is a transmembrane molecule, it has been used in antibody-mediated positive selection strategies for endothelial cell isolation. However, expression of the

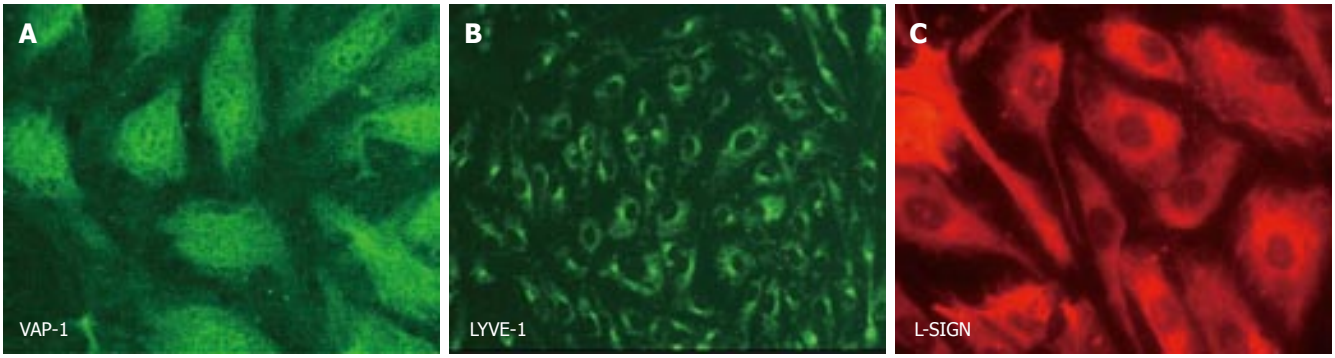


Figure 3 Cultured human hepatic sinusoidal endothelial cells stain positively with antibody directed against 'non-classical endothelial' phenotypic markers. Images represent immunofluorescent staining of cultured cells using specific primary antibodies in an indirect fluorescent protocol. Expression of VAP-1 (**A**) and LYVE-1 (**B**) are visualised with a FITC-conjugated secondary antibody (green), whilst positive staining for L-SIGN (**C**) is visualised using a Texas Red-labelled secondary antibody.

molecule is not restricted to endothelial cells[65], and in the liver, expression has been reported in both stellate cells and myofibroblasts[67]. We have demonstrated that CD105 is expressed on hepatic sinusoidal endothelial cells (Figure 2) but again emphasise that this is not a tissue- or cell lineage-specific marker and urge caution when using it as a phenotypic identifier.

NEWER MARKERS OF ENDOTHELIAL PHENOTYPE RELATE TO THE FUNCTIONS OF HSEC WITHIN THE LIVER MICRO-ENVIRONMENT

As well as exhibiting features characteristic of all endothelial cells, hepatic sinusoidal endothelial cells fulfil many specific features within the liver environment. These include providing a barrier to minimise access of blood-borne material into the parenchyma and specific protein/antigen uptake and presentation. The embryonic origins of these cells and their similarity with lymphatic endothelial cells (see later) mean that they express several markers that are not present on vascular endothelium and these can be used to distinguish them from other endothelial cells. Thus, it is possible to use protein markers related to these specific origins and functions to confirm the phenotype of HSEC *in vitro*.

Scavenger functions/lipid uptake functions of EC

The exposure of sinusoidal endothelial cells to blood originating from both the systemic circulation and the gut means that HSEC are strategically situated to remove and recycle blood-borne proteins and lipids. In combination with Kupffer cells, HSEC constitute the most powerful scavenger system in the body[68]. The uptake of solutes is facilitated by the presence of fenestrae, the lack of a classical basement membrane and the expression of multiple scavenger receptors that allow them to bind and take up specific classes of molecules. These properties facilitate bidirectional transfer of materials to the parenchyma. Many of the scavenger receptor proteins can be used to determine the phenotype of HSEC.

The link family of proteins has recently been described as scavenger receptors responsible for clearance of a variety of proteins, including advanced glycation end products, modified LDL and bacteria[69]. Two members of this protein family, Stabilin-1 and -2, are constitutively expressed by hepatic sinusoidal endothelial cells[69,70]. Stabilin-2 is the major lymph node and liver hyaluronan and glycosaminoglycan scavenger receptor whilst Stabilin-1 (also called Feel-1 or CLEVER-1[71-73] is a more promiscuous scavenger receptor. In common with many other scavenger-type receptors, these proteins are present on sinusoidal endothelial cells in spleen and lymph node as well as the liver. Most of the scavenger functions assigned to this molecule relate to endocytosis of hyaluronic acid, acetylated LDL and glycation end products, but there is also evidence to support roles for Stabilin-1 in leukocyte adhesion and tumour metastasis[69,74]. Another member of the link protein family is LYVE-1, an endothelial hyaluronan receptor predominantly restricted to lymphatic endothelial cells (reviewed in[75]). Putative functions for LYVE-1 include the uptake of hyaluronic acid and regulation of leukocyte adhesion or migration within the lymphatic circulation. Interestingly, hepatic sinusoidal endothelial cells also express LYVE-1 constitutively[76] (Figure 3) with evidence of a zonal distribution, the highest levels being detected in acinar zone 2. This hyaluronan receptor is present on both normal and diseased human HSEC, although lower levels are observed in cirrhosis and the protein is absent in HCC[76]. Expression is also seen on portal-associated lymphatics in chronic liver disease[58,76].

The liver is the major site for synthesis and metabolism of cholesterol and scavenger receptors of class A (SR-A) on both KC and HSEC[77] are responsible for the uptake of oxidised/acetylated LDL, which is subsequently passed on to hepatocytes. Another HDL/LDL receptor, CD36[78], also known as GPIV, is expressed at high levels on platelets, monocyte/macrophages and vascular endothelial cells. In the liver, CD36 is strongly expressed on sinusoidal endothelial cells[79] where it fulfills multiple functions including acting as a scavenger receptor for oxidised lipid[80] and as an adhesion receptor for red blood cells infected with malarial parasite[81]. There are two alternatively spliced members of the scavenger receptor B family (SR-BI and -BⅡ). Scavenger receptor-B1 is expressed by HSEC and

is responsible for the uptake of HDL cholesterol esters to liver parenchymal cells and also acts as a coreceptor for HCV infection[82].

The calcium-dependent C-type lectins, Dendritic Cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN) or CD209 and the related molecule Liver/lymph node-specific intercellular adhesion molecule-3-grabbing nonintegrin (L-SIGN) or CD209L (Figure 3), are constitutively expressed on hepatic endothelial cells[83]. DC-SIGN is expressed at high levels on myeloid dendritic cells in tissues where it interacts with ICAM-3 on T cells as part of the immunological synapse as well as being an attachment factor for HCV, ebola virus, CMV, HIV and other lentiviruses (reviewed in[84,85]). We have recently reported that DC-SIGN is present on hepatic sinusoidal endothelial cells and that expression is increased in response to treatment with cytokines including IL-4[86]. DC-SIGN on endothelial cells acts as an attachment factor for HCV but does not mediate HCV entry directly but rather enhances infection of hepatocytes in trans. DC-SIGN is absent from most other vascular beds, although it has been reported on a brain microvascular cell line[87].

Liver/lymph node-specific ICAM-3-grabbing integrin (L-SIGN), otherwise known as DC-SIGN-related (DC SIGNR) (CD209L), shares 77% amino acid homology with DC-SIGN. Like DC-SIGN it can also bind ICAM-3, HIV and HCV. L-SIGN is strongly and constitutively expressed on sinusoidal endothelial cells in the liver and on endothelium in lymph nodes but not on DCs or on endothelium in other tissues. L-SIGN is thus an excellent marker of liver endothelium[44,88]. A related molecule, Liver and Lymph node Sinusoidal Endothelial C-type lectin (LSECtin) is also expressed on sinusoidal endothelial cells and has recently been demonstrated to mediate attachment of filovirus and coronavirus particles[89]. Thus sinusoidal endothelial cells[90] can bind a wide variety of pathogens after which they pass hepatotropic viruses on to adherent hepatocytes in trans[91] thereby concentrating viral pathogens within the liver.

Antigen presentation by HSEC

As well as being equipped with scavenger receptors that facilitate efficient uptake of viruses and potential antigens, HSEC also have the ability to phagocytose particles and to present antigen to lymphocytes(reviewed in [92-94]). There is evidence that such interactions are important for generating immunological tolerance to gut-derived antigens although recent work suggests that local antigen presentation cannot explain liver tolerance and that, on the contrary, the liver may be an excellent priming site for naive CD8+ T cells[95]. Antigen presentation is facilitated by the expression of MHC class I and Ⅱ[96,97] molecules together with co-stimulatory molecules such as CD40 and more contentiously CD80 and CD86[98]. These receptors are upregulated on HSEC in fulminant liver failure[99] and may contribute to disease pathogenesis by allowing ongoing presentation of stimulatory antigen. HSEC also express the mannose receptor (Figure 1), a 175 kDa transmembrane glycosylated protein involved in uptake of Ag by both DCs and HSEC[97]. Competitive inhibition of this receptor by mannan reduces antigen-specific T cell activation by murine HSEC[97].

Similarities between lymphatic endothelial cells and sinusoidal endothelium

Both the liver and pancreas develop from buds of the embryonic endoderm[100], however, the vasculature components of the liver have distinct origins. The portal vessels are derived from viteine veins whereas the sinusoids develop from the capillary vessels of the septum transversum and acquire their distinctive fenestrated phenotype by wk 20 of gestation (reviewed in[21]). From this point onward, sinusoidal endothelial cells remain functionally and phenotypically distinct from the other vascular endothelial cells in the liver and express several receptors that are otherwise confined to lymphatic endothelial cells that are derived from buds from the cardinal vein. Hence, both lymphatic and sinusoidal endothelial cells have minimal basement membranes, loosely organised cell junctions and constitutively express LYVE-1 and VAP-1 (SSAO/AOC3) but lack CD34 (reviewed in[101]). VAP-1 is a type Ⅱ transmembrane protein that can support leukocyte adhesion *via* interactions with sialic acid rich side chains. It is also an amine oxidase and enzyme activity is also involved in regulating leukocyte adhesion and transmigration[101]. VAP-1 is expressed on all vascular compartments within the liver (Figures 1 and 2) where it supports the adhesion and transmigration of leukocytes[10,102]. The only extrahepatic site where VAP-1 is constitutively expressed at high levels is endothelial cells in high endothelial venules within lymph nodes[103] where again it is proposed to have a role directing the adhesion of lymphocyte populations[104]. Similarly both lymphatic endothelium and HSEC express the Reeler gene product Reelin[105]. Reelin is a secreted glycoprotein with roles in embryonic development and organisation. Expression is restricted during embryogenesis but in the adult organism high levels are detected on lymphatic endothelial cells and within the sinusoids localised either to stellate cells[106] or HSEC[105]. This has led to the hypothesis that reelin may be involved in the regulation of lymphoangiogenesis or regulation of lymphatic endothelial phenotype and thus may have similar roles within the liver sinusoids.

Thus there are many similarities between HSEC and lymphatic endothelial cells and some antigens originally defined on lymphatic endothelium can also be used to differentiate between HSEC and vascular endothelial cells in the liver. It is possible to exclude contamination with lymphatic EC in HSEC cultures on the basis that LYVE-1 positive HSEC do not express PROX -1, a transcription factor found exclusively in lymphatic EC[107].

CONCLUSIONS

Cultures of endothelial cells are valuable tools to investigate mechanisms of liver physiology and pathophysiology *in vitro*. However, the study of endothelial cells *in vitro* is complicated by the marked heterogeneity of endothelial cells between and within different organs and the tendency for cells to lose tissue-specific markers

when cultured *in vitro*. Although all endothelial cells share some characteristic features (as described in the first part of this review), there is a need for specific markers or combinations of markers that define distinct populations of endothelial cells. To date, the study of HSEC *in vitro* has been hampered by the lack of specific markers that can conclusively identify these cells and discriminate them from vascular or lymphatic endothelial cells. To some extent this remains the case, since many classical endothelial markers are widely expressed (Table 1) and there is considerable reported variability in detection of phenotypic markers between animal and human systems (eg CD31 and vWF). To date there is no known single molecule that is only expressed on hepatic sinusoidal and no other type of endothelia. However, the increasing knowledge of endothelial receptors is providing us with a larger and better defined set of phenotypic makers. In addition to their use in phenotyping or sorting/selecting specific endothelial cells for culture, receptors that show tissue-specific expression provide clues to specific functions of the cells being studied. Examples of this are the large number of scavenger receptors expressed by HSEC. Cultured HSEC do exhibit some useful identifying features, however. Very low passage cells retain fenestrations *in vitro* for a short time but these rapidly disappear within a passage or two in culture[8,16], as does expression of VAP-1[10]. Apart from these changes, however, the cells remain relatively phenotypically stable for 7-8 passages[8] and can be identified by expression of CD31, LYVE-1, DC-SIGNR(L-SIGN), Stabilin-1 and lack of CD34 and PROX-1[107]. These markers confirm endothelial identity, while excluding vascular and lymphatic endothelial contamination and in conjunction with markers to exclude cells of leukocyte origin can be used to confirm the sinusoidal nature of the cells.

REFERENCES

1 **Matsumoto T**, Kawakami M. The unit-concept of hepatic parenchyma--a re-examination based on angioarchitectural studies. *Acta Pathol Jpn* 1982; **32** Suppl 2: 285-314
2 **Takasaki S**, Hano H. Three-dimensional observations of the human hepatic artery (Arterial system in the liver). *J Hepatol* 2001; **34**: 455-466
3 **Braet F**, Wisse E. Structural and functional aspects of liver sinusoidal endothelial cell fenestrae: a review. *Comp Hepatol* 2002; **1**: 1
4 **Do H**, Healey JF, Waller EK, Lollar P. Expression of factor VIII by murine liver sinusoidal endothelial cells. *J Biol Chem* 1999; **274**: 19587-19592
5 **Vidal-Vanaclocha F**, Rocha M, Asumendi A, Barberá-Guillem E. Isolation and enrichment of two sublobular compartment-specific endothelial cell subpopulations from liver sinusoids. *Hepatology* 1993; **18**: 328-339
6 **Yamane A**, Seetharam L, Yamaguchi S, Gotoh N, Takahashi T, Neufeld G, Shibuya M. A new communication system between hepatocytes and sinusoidal endothelial cells in liver through vascular endothelial growth factor and Flt tyrosine kinase receptor family (Flt-1 and KDR/Flk-1). *Oncogene* 1994; **9**: 2683-2690
7 **Knolle PA**, Germann T, Treichel U, Uhrig A, Schmitt E, Hegenbarth S, Lohse AW, Gerken G. Endotoxin down-regulates T cell activation by antigen-presenting liver sinusoidal endothelial cells. *J Immunol* 1999; **162**: 1401-1407
8 **Daneker GW**, Lund SA, Caughman SW, Swerlick RA, Fischer AH, Staley CA, Ades EW. Culture and characterization of sinusoidal endothelial cells isolated from human liver. *In Vitro Cell Dev Biol Anim* 1998; **34**: 370-377
9 **Gerlach JC**, Zeilinger K, Spatkowski G, Hentschel F, Schnoy N, Kolbeck S, Schindler RK, Neuhaus P. Large-scale isolation of sinusoidal endothelial cells from pig and human liver. *J Surg Res* 2001; **100**: 39-45
10 **Lalor PF**, Edwards S, McNab G, Salmi M, Jalkanen S, Adams DH. Vascular adhesion protein-1 mediates adhesion and transmigration of lymphocytes on human hepatic endothelial cells. *J Immunol* 2002; **169**: 983-992
11 **Katz SC**, Pillarisetty VG, Bleier JI, Shah AB, DeMatteo RP. Liver sinusoidal endothelial cells are insufficient to activate T cells. *J Immunol* 2004; **173**: 230-235
12 **Gomez DE**, Hartzler JL, Corbitt RH, Nason AM, Thorgeirsson UP. Immunomagnetic separation as a final purification step of liver endothelial cells. *In Vitro Cell Dev Biol Anim* 1993; **29A**: 451-455
13 **Sawyer RT**, Garner RE, Hudson JA. Effect of lectins on hepatic clearance and killing of Candida albicans by the isolated perfused mouse liver. *Infect Immun* 1992; **60**: 1041-1046
14 **Scoazec JY**, Feldmann G. Both macrophages and endothelial cells of the human hepatic sinusoid express the CD4 molecule, a receptor for the human immunodeficiency virus. *Hepatology* 1990; **12**: 505-510
15 **Scoazec JY**, Racine L, Couvelard A, Flejou JF, Feldmann G. Endothelial cell heterogeneity in the normal human liver acinus: in situ immunohistochemical demonstration. *Liver* 1994; **14**: 113-123
16 **DeLeve LD**, Wang X, Hu L, McCuskey MK, McCuskey RS. Rat liver sinusoidal endothelial cell phenotype is maintained by paracrine and autocrine regulation. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G757-G763
17 **Módis L**, Martinez-Hernandez A. Hepatocytes modulate the hepatic microvascular phenotype. *Lab Invest* 1991; **65**: 661-670
18 **Edwards S**, Lalor PF, Nash GB, Rainger GE, Adams DH. Lymphocyte traffic through sinusoidal endothelial cells is regulated by hepatocytes. *Hepatology* 2005; **41**: 451-459
19 **Ghitescu L**, Robert M. Diversity in unity: the biochemical composition of the endothelial cell surface varies between the vascular beds. *Microsc Res Tech* 2002; **57**: 381-389
20 **Jackson DG**. The lymphatics revisited: new perspectives from the hyaluronan receptor LYVE-1. *Trends Cardiovasc Med* 2003; **13**: 1-7
21 **Gouysse G**, Couvelard A, Frachon S, Bouvier R, Nejjari M, Dauge MC, Feldmann G, Hénin D, Scoazec JY. Relationship between vascular development and vascular differentiation during liver organogenesis in humans. *J Hepatol* 2002; **37**: 730-740
22 **Steffan AM**, Pereira CA, Bingen A, Valle M, Martin JP, Koehren F, Royer C, Gendrault JL, Kirn A. Mouse hepatitis virus type 3 infection provokes a decrease in the number of sinusoidal endothelial cell fenestrae both in vivo and in vitro. *Hepatology* 1995; **22**: 395-401
23 **Vidal-Vanaclocha F**, Barberá-Guillem E. Fenestration patterns in endothelial cells of rat liver sinusoids. *J Ultrastruct Res* 1985; **90**: 115-123
24 **Horn T**, Christoffersen P, Henriksen JH. Alcoholic liver injury: defenestration in noncirrhotic livers--a scanning electron microscopic study. *Hepatology* 1987; **7**: 77-82
25 **Mak KM**, Lieber CS. Alterations in endothelial fenestrations in liver sinusoids of baboons fed alcohol: a scanning electron microscopic study. *Hepatology* 1984; **4**: 386-391
26 **Le Couteur DG**, Fraser R, Hilmer S, Rivory LP, McLean AJ. The hepatic sinusoid in aging and cirrhosis: effects on hepatic substrate disposition and drug clearance. *Clin Pharmacokinet* 2005; **44**: 187-200
27 **Xu B**, Broome U, Uzunel M, Nava S, Ge X, Kumagai-Braesch M, Hultenby K, Christensson B, Ericzon BG, Holgersson J, Sumitran-Holgersson S. Capillarization of hepatic sinusoid by liver endothelial cell-reactive autoantibodies in patients with cirrhosis and chronic hepatitis. *Am J Pathol* 2003; **163**: 1275-1289

28 **Chen J**, Braet F, Brodsky S, Weinstein T, Romanov V, Noiri E, Goligorsky MS. VEGF-induced mobilization of caveolae and increase in permeability of endothelial cells. *Am J Physiol Cell Physiol* 2002; **282**: C1053-C1063

29 **Funyu J**, Mochida S, Inao M, Matsui A, Fujiwara K. VEGF can act as vascular permeability factor in the hepatic sinusoids through upregulation of porosity of endothelial cells. *Biochem Biophys Res Commun* 2001; **280**: 481-485

30 **Krause P**, Markus PM, Schwartz P, Unthan-Fechner K, Pestel S, Fandrey J, Probst I. Hepatocyte-supported serum-free culture of rat liver sinusoidal endothelial cells. *J Hepatol* 2000; **32**: 718-726

31 **Yokomori H**, Oda M, Yoshimura K, Nagai T, Ogi M, Nomura M, Ishii H. Vascular endothelial growth factor increases fenestral permeability in hepatic sinusoidal endothelial cells. *Liver Int* 2003; **23**: 467-475

32 **Watanabe Y**, Dvorak HF. Vascular permeability factor/vascular endothelial growth factor inhibits anchorage-disruption-induced apoptosis in microvessel endothelial cells by inducing scaffold formation. *Exp Cell Res* 1997; **233**: 340-349

33 **Esser S**, Wolburg K, Wolburg H, Breier G, Kurzchalia T, Risau W. Vascular endothelial growth factor induces endothelial fenestrations in vitro. *J Cell Biol* 1998; **140**: 947-959

34 **Scoazec JY**, Feldmann G. The cell adhesion molecules of hepatic sinusoidal endothelial cells. *J Hepatol* 1994; **20**: 296-300

35 **Hahn E**, Wick G, Pencev D, Timpl R. Distribution of basement membrane proteins in normal and fibrotic human liver: collagen type IV, laminin, and fibronectin. *Gut* 1980; **21**: 63-71

36 **Newton JP**, Hunter AP, Simmons DL, Buckley CD, Harvey DJ. CD31 (PECAM-1) exists as a dimer and is heavily N-glycosylated. *Biochem Biophys Res Commun* 1999; **261**: 283-291

37 **Neubauer K**, Ritzel A, Saile B, Ramadori G. Decrease of platelet-endothelial cell adhesion molecule 1-gene-expression in inflammatory cells and in endothelial cells in the rat liver following CCl(4)-administration and in vitro after treatment with TNFalpha. *Immunol Lett* 2000; **74**: 153-164

38 **Medina J**, Sanz-Cameno P, García-Buey L, Martín-Vílchez S, López-Cabrera M, Moreno-Otero R. Evidence of angiogenesis in primary biliary cirrhosis: an immunohistochemical descriptive study. *J Hepatol* 2005; **42**: 124-131

39 **Unger RE**, Krump-Konvalinkova V, Peters K, Kirkpatrick CJ. In vitro expression of the endothelial phenotype: comparative study of primary isolated cells and cell lines, including the novel cell line HPMEC-ST1.6R. *Microvasc Res* 2002; **64**: 384-397

40 **Yamamoto K**, de Waard V, Fearns C, Loskutoff DJ. Tissue distribution and regulation of murine von Willebrand factor gene expression in vivo. *Blood* 1998; **92**: 2791-2801

41 **Harrison RL**, Boudreau R. Human hepatic sinusoidal endothelial cells in culture produce von Willebrand factor and contain Weibel-Palade bodies. *Liver* 1989; **9**: 242-249

42 **Lenzi R**, Alpini G, Liu MH, Rand JH, Tavoloni N. von Willebrand factor antigen is not an accurate marker of rat and guinea pig liver endothelial cells. *Liver* 1990; **10**: 372-379

43 **Wisse E**. An ultrastructural characterization of the endothelial cell in the rat liver sinusoid under normal and various experimental conditions, as a contribution to the distinction between endothelial and Kupffer cells. *J Ultrastruct Res* 1972; **38**: 528-562

44 **Pöhlmann S**, Soilleux EJ, Baribaud F, Leslie GJ, Morris LS, Trowsdale J, Lee B, Coleman N, Doms RW. DC-SIGNR, a DC-SIGN homologue expressed in endothelial cells, binds to human and simian immunodeficiency viruses and activates infection in trans. *Proc Natl Acad Sci USA* 2001; **98**: 2670-2675

45 **Fukuda Y**, Nagura H, Imoto M, Koyama Y. Immunohistochemical studies on structural changes of the hepatic lobules in chronic liver diseases. *Am J Gastroenterol* 1986; **81**: 1149-1155

46 **Tedder TF**, Steeber DA, Chen A, Engel P. The selectins: vascular adhesion molecules. *FASEB J* 1995; **9**: 866-873

47 **Lalor PF**, Shields P, Grant A, Adams DH. Recruitment of lymphocytes to the human liver. *Immunol Cell Biol* 2002; **80**: 52-64

48 **Khatib AM**, Kontogiannea M, Fallavollita L, Jamison B, Meterissian S, Brodt P. Rapid induction of cytokine and E-selectin expression in the liver in response to metastatic tumor cells. *Cancer Res* 1999; **59**: 1356-1361

49 **Wong J**, Johnston B, Lee SS, Bullard DC, Smith CW, Beaudet AL, Kubes P. A minimal role for selectins in the recruitment of leukocytes into the inflamed liver microvasculature. *J Clin Invest* 1997; **99**: 2782-2790

50 **Makondo K**, Kimura K, Kitamura T, Yamaji D, Dong Jung B, Shibata H, Saito M. Hepatocyte growth factor/scatter factor suppresses TNF-alpha-induced E-selectin expression in human umbilical vein endothelial cells. *Biochim Biophys Acta* 2004; **1644**: 9-15

51 **Holthöfer H**, Virtanen I, Kariniemi AL, Hormia M, Linder E, Miettinen A. Ulex europaeus I lectin as a marker for vascular endothelium in human tissues. *Lab Invest* 1982; **47**: 60-66

52 **Hattori M**, Fukuda Y, Imoto M, Koyama Y, Nakano I, Urano F. Histochemical properties of vascular and sinusoidal endothelial cells in liver diseases. *Gastroenterol Jpn* 1991; **26**: 336-343

53 **De Rijke YB**, Biessen EA, Vogelezang CJ, van Berkel TJ. Binding characteristics of scavenger receptors on liver endothelial and Kupffer cells for modified low-density lipoproteins. *Biochem J* 1994; **304** (Pt 1): 69-73

54 **Ling W**, Lougheed M, Suzuki H, Buchan A, Kodama T, Steinbrecher UP. Oxidized or acetylated low density lipoproteins are rapidly cleared by the liver in mice with disruption of the scavenger receptor class A type I/II gene. *J Clin Invest* 1997; **100**: 244-252

55 **Couvelard A**, Scoazec JY, Feldmann G. Expression of cell-cell and cell-matrix adhesion proteins by sinusoidal endothelial cells in the normal and cirrhotic human liver. *Am J Pathol* 1993; **143**: 738-752

56 **Cui S**, Hano H, Sakata A, Harada T, Liu T, Takai S, Ushigome S. Enhanced CD34 expression of sinusoid-like vascular endothelial cells in hepatocellular carcinoma. *Pathol Int* 1996; **46**: 751-756

57 **Frachon S**, Gouysse G, Dumortier J, Couvelard A, Nejjari M, Mion F, Berger F, Paliard P, Boillot O, Scoazec JY. Endothelial cell marker expression in dysplastic lesions of the liver: an immunohistochemical study. *J Hepatol* 2001; **34**: 850-857

58 **Grant AJ**, Goddard S, Ahmed-Choudhury J, Reynolds G, Jackson DG, Briskin M, Wu L, Hübscher SG, Adams DH. Hepatic expression of secondary lymphoid chemokine (CCL21) promotes the development of portal-associated lymphoid tissue in chronic inflammatory liver disease. *Am J Pathol* 2002; **160**: 1445-1455

59 **Schlingemann RO**, Dingjan GM, Emeis JJ, Blok J, Warnaar SO, Ruiter DJ. Monoclonal antibody PAL-E specific for endothelium. *Lab Invest* 1985; **52**: 71-76

60 **Niemelä H**, Elima K, Henttinen T, Irjala H, Salmi M, Jalkanen S. Molecular identification of PAL-E, a widely used endothelial-cell marker. *Blood* 2005; **106**: 3405-3409

61 **Xu B**, deWaal RM, Mor-Vaknin N, Hibbard C, Markovitz DM, Kahn ML. The endothelial cell-specific antibody PAL-E identifies a secreted form of vimentin in the blood vasculature. *Mol Cell Biol* 2004; **24**: 9198-9206

62 **Stan RV**, Kubitza M, Palade GE. PV-1 is a component of the fenestral and stomatal diaphragms in fenestrated endothelia. *Proc Natl Acad Sci USA* 1999; **96**: 13203-13207

63 **Medina J**, Caveda L, Sanz-Cameno P, Arroyo AG, Martín-Vílchez S, Majano PL, García-Buey L, Sánchez-Madrid F, Moreno-Otero R. Hepatocyte growth factor activates endothelial proangiogenic mechanisms relevant in chronic hepatitis C-associated neoangiogenesis. *J Hepatol* 2003; **38**: 660-667

64 **Palmeri D**, van Zante A, Huang CC, Hemmerich S, Rosen SD. Vascular endothelial junction-associated molecule, a novel member of the immunoglobulin superfamily, is localized to intercellular boundaries of endothelial cells. *J Biol Chem* 2000; **275**: 19139-19145

65 **Duff SE**, Li C, Garland JM, Kumar S. CD105 is important for angiogenesis: evidence and potential applications. *FASEB J* 2003; **17**: 984-992

66 **Saad RS**, Liu YL, Nathan G, Celebrezze J, Medich D, Silverman JF. Endoglin (CD105) and vascular endothelial growth factor as prognostic markers in colorectal cancer. *Mod Pathol*

2004; **17**: 197-203
67 **Meurer SK**, Tihaa L, Lahme B, Gressner AM, Weiskirchen R. Identification of endoglin in rat hepatic stellate cells: new insights into transforming growth factor beta receptor signaling. *J Biol Chem* 2005; **280**: 3078-3087
68 **Elvevold KH**, Nedredal GI, Revhaug A, Smedsrød B. Scavenger properties of cultivated pig liver endothelial cells. *Comp Hepatol* 2004; **3**: 4
69 **Prevo R**, Banerji S, Ni J, Jackson DG. Rapid plasma membrane-endosomal trafficking of the lymph node sinus and high endothelial venule scavenger receptor/homing receptor stabilin-1 (FEEL-1/CLEVER-1). *J Biol Chem* 2004; **279**: 52580-52592
70 **Hansen B**, Longati P, Elvevold K, Nedredal GI, Schledzewski K, Olsen R, Falkowski M, Kzhyshkowska J, Carlsson F, Johansson S, Smedsrød B, Goerdt S, Johansson S, McCourt P. Stabilin-1 and stabilin-2 are both directed into the early endocytic pathway in hepatic sinusoidal endothelium via interactions with clathrin/AP-2, independent of ligand binding. *Exp Cell Res* 2005; **303**: 160-173
71 **Politz O**, Gratchev A, McCourt PA, Schledzewski K, Guillot P, Johansson S, Svineng G, Franke P, Kannicht C, Kzhyshkowska J, Longati P, Velten FW, Johansson S, Goerdt S. Stabilin-1 and -2 constitute a novel family of fasciclin-like hyaluronan receptor homologues. *Biochem J* 2002; **362**: 155-164
72 **Tamura Y**, Adachi H, Osuga J, Ohashi K, Yahagi N, Sekiya M, Okazaki H, Tomita S, Iizuka Y, Shimano H, Nagai R, Kimura S, Tsujimoto M, Ishibashi S. FEEL-1 and FEEL-2 are endocytic receptors for advanced glycation end products. *J Biol Chem* 2003; **278**: 12613-12617
73 **Irjala H**, Elima K, Johansson EL, Merinen M, Kontula K, Alanen K, Grenman R, Salmi M, Jalkanen S. The same endothelial receptor controls lymphocyte traffic both in vascular and lymphatic vessels. *Eur J Immunol* 2003; **33**: 815-824
74 **Salmi M**, Koskinen K, Henttinen T, Elima K, Jalkanen S. CLEVER-1 mediates lymphocyte transmigration through vascular and lymphatic endothelium. *Blood* 2004; **104**: 3849-3857
75 **Jackson DG**. Biology of the lymphatic marker LYVE-1 and applications in research into lymphatic trafficking and lymphangiogenesis. *APMIS* 2004; **112**: 526-538
76 **Mouta Carreira C**, Nasser SM, di Tomaso E, Padera TP, Boucher Y, Tomarev SI, Jain RK. LYVE-1 is not restricted to the lymph vessels: expression in normal liver blood sinusoids and down-regulation in human liver cancer and cirrhosis. *Cancer Res* 2001; **61**: 8079-8084
77 **van Oosten M**, van de Bilt E, van Berkel TJ, Kuiper J. New scavenger receptor-like receptors for the binding of lipopolysaccharide to liver endothelial and Kupffer cells. *Infect Immun* 1998; **66**: 5107-5112
78 **Malerød L,** Juvet K, Gjøen T, Berg T. The expression of scavenger receptor class B, type I (SR-BI) and caveolin-1 in parenchymal and nonparenchymal liver cells. *Cell Tissue Res* 2002; **307**: 173-180
79 **Scoazec JY**, Feldmann G. In situ immunophenotyping study of endothelial cells of the human hepatic sinusoid: results and functional implications. *Hepatology* 1991; **14**: 789-797
80 **Ohgami N**, Nagai R, Ikemoto M, Arai H, Kuniyasu A, Horiuchi S, Nakayama H. CD36, a member of class B scavenger receptor family, is a receptor for advanced glycation end products. *Ann N Y Acad Sci* 2001; **947**: 350-355
81 **Mota MM**, Jarra W, Hirst E, Patnaik PK, Holder AA. Plasmodium chabaudi-infected erythrocytes adhere to CD36 and bind to microvascular endothelial cells in an organ-specific way. *Infect Immun* 2000; **68**: 4135-4144
82 **Bartosch B**, Vitelli A, Granier C, Goujon C, Dubuisson J, Pascale S, Scarselli E, Cortese R, Nicosia A, Cosset FL. Cell entry of hepatitis C virus requires a set of co-receptors that include the CD81 tetraspanin and the SR-B1 scavenger receptor. *J Biol Chem* 2003; **278**: 41624-41630
83 **Engering A**, van Vliet SJ, Hebeda K, Jackson DG, Prevo R, Singh SK, Geijtenbeek TB, van Krieken H, van Kooyk Y. Dynamic populations of dendritic cell-specific ICAM-3 grabbing nonintegrin-positive immature dendritic cells and liver/lymph node-specific ICAM-3 grabbing nonintegrin-positive endothelial cells in the outer zones of the paracortex of human lymph nodes. *Am J Pathol* 2004; **164**: 1587-1595
84 **van Kooyk Y**, Geijtenbeek TB. DC-SIGN: escape mechanism for pathogens. *Nat Rev Immunol* 2003; **3**: 697-709
85 **Cocquerel L**, Voisset C, Dubuisson J. Hepatitis C virus entry: potential receptors and their biological functions. *J Gen Virol* 2006; **87**: 1075-1084
86 **Bashirova AA**, Geijtenbeek TB, van Duijnhoven GC, van Vliet SJ, Eilering JB, Martin MP, Wu L, Martin TD, Viebig N, Knolle PA, KewalRamani VN, van Kooyk Y, Carrington M. A dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN)-related protein is highly expressed on human liver sinusoidal endothelial cells and promotes HIV-1 infection. *J Exp Med* 2001; **193**: 671-678
87 **Mukhtar M**, Harley S, Chen P, BouHamdan M, Patel C, Acheampong E, Pomerantz RJ. Primary isolated human brain microvascular endothelial cells express diverse HIV/SIV-associated chemokine coreceptors and DC-SIGN and L-SIGN. *Virology* 2002; **297**: 78-88
88 **Cormier EG**, Durso RJ, Tsamis F, Boussemart L, Manix C, Olson WC, Gardner JP, Dragic T. L-SIGN (CD209L) and DC-SIGN (CD209) mediate transinfection of liver cells by hepatitis C virus. *Proc Natl Acad Sci USA* 2004; **101**: 14067-14072
89 **Gramberg T**, Hofmann H, Möller P, Lalor PF, Marzi A, Geier M, Krumbiegel M, Winkler T, Kirchhoff F, Adams DH, Becker S, Münch J, Pöhlmann S. LSECtin interacts with filovirus glycoproteins and the spike protein of SARS coronavirus. *Virology* 2005; **340**: 224-236
90 **Housset C**, Lamas E, Courgnaud V, Boucher O, Girard PM, Marche C, Brechot C. Presence of HIV-1 in human parenchymal and non-parenchymal liver cells in vivo. *J Hepatol* 1993; **19**: 252-258
91 **Breiner KM**, Schaller H, Knolle PA. Endothelial cell-mediated uptake of a hepatitis B virus: a new concept of liver targeting of hepatotropic microorganisms. *Hepatology* 2001; **34**: 803-808
92 **Knolle PA**, Limmer A. Control of immune responses by savenger liver endothelial cells. *Swiss Med Wkly* 2003; **133**: 501-506
93 **Onoe T**, Ohdan H, Tokita D, Shishida M, Tanaka Y, Hara H, Zhou W, Ishiyama K, Mitsuta H, Ide K, Asahara T. Liver sinusoidal endothelial cells tolerize T cells across MHC barriers in mice. *J Immunol* 2005; **175**: 139-146
94 **Tokita D**, Ohdan H, Onoe T, Hara H, Tanaka Y, Asahara T. Liver sinusoidal endothelial cells contribute to alloreactive T-cell tolerance induced by portal venous injection of donor splenocytes. *Transpl Int* 2005; **18**: 237-245
95 **Klein I**, Crispe IN. Complete differentiation of CD8+ T cells activated locally within the transplanted liver. *J Exp Med* 2006; **203**: 437-447
96 **Lohse AW**, Knolle PA, Bilo K, Uhrig A, Waldmann C, Ibe M, Schmitt E, Gerken G, Meyer Zum Büschenfelde KH. Antigen-presenting function and B7 expression of murine sinusoidal endothelial cells and Kupffer cells. *Gastroenterology* 1996; **110**: 1175-1181
97 **Knolle PA**, Uhrig A, Hegenbarth S, Loser E, Schmitt E, Gerken G, Lohse AW. IL-10 down-regulates T cell activation by antigen-presenting liver sinusoidal endothelial cells through decreased antigen uptake via the mannose receptor and lowered surface expression of accessory molecules. *Clin Exp Immunol* 1998; **114**: 427-433
98 **Ahmed-Choudhury J**, Russell CL, Randhawa S, Young LS, Adams DH, Afford SC. Differential induction of nuclear factor-kappaB and activator protein-1 activity after CD40 ligation is associated with primary human hepatocyte apoptosis or intrahepatic endothelial cell proliferation. *Mol Biol Cell* 2003; **14**: 1334-1345
99 **Leifeld L**, Trautwein C, Dumoulin FL, Manns MP, Sauerbruch T, Spengler U. Enhanced expression of CD80 (B7-1), CD86 (B7-2), and CD40 and their ligands CD28 and CD154 in fulminant hepatic failure. *Am J Pathol* 1999; **154**: 1711-1720
100 **Lammert E**, Cleaver O, Melton D. Role of endothelial cells in

early pancreas and liver development. *Mech Dev* 2003; **120**: 59-64
101 **Salmi M**, Jalkanen S. Cell-surface enzymes in control of leukocyte trafficking. *Nat Rev Immunol* 2005; **5**: 760-771
102 **Bonder CS**, Norman MU, Swain MG, Zbytnuik LD, Yamanouchi J, Santamaria P, Ajuebor M, Salmi M, Jalkanen S, Kubes P. Rules of recruitment for Th1 and Th2 lymphocytes in inflamed liver: a role for alpha-4 integrin and vascular adhesion protein-1. *Immunity* 2005; **23**: 153-163
103 **Salmi M**, Kalimo K, Jalkanen S. Induction and function of vascular adhesion protein-1 at sites of inflammation. *J Exp Med* 1993; **178**: 2255-2260
104 **Salmi M**, Hellman J, Jalkanen S. The role of two distinct endothelial molecules, vascular adhesion protein-1 and peripheral lymph node addressin, in the binding of lymphocyte subsets to human lymph nodes. *J Immunol* 1998; **160**: 5629-5636
105 **Ikeda Y**, Terashima T. Expression of reelin, the gene responsible for the reeler mutation, in embryonic development and adulthood in the mouse. *Dev Dyn* 1997; **210**: 157-172
106 **Samama B**, Boehm N. Reelin immunoreactivity in lymphatics and liver during development and adult life. *Anat Rec A Discov Mol Cell Evol Biol* 2005; **285**: 595-599
107 **Hong YK**, Detmar M. Prox1, master regulator of the lymphatic vasculature phenotype. *Cell Tissue Res* 2003; **314**: 85-92

S- Editor Wang J **L- Editor** Lutze M **E- Editor** Ma WH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5440-5446
World Journal of Gastroenterology ISSN 1007-9327

EDITORIAL

Sporadic versus hereditary gastrinomas of the duodenum and pancreas: Distinct clinico-pathological and epidemiological features

Martin Anlauf, Nele Garbrecht, Tobias Henopp, Anja Schmitt, Regina Schlenger, Andreas Raffel, Markus Krausch, Oliver Gimm, Claus F Eisenberger, Wolfram T Knoefel, Henning Dralle, Paul Komminoth, Philipp U Heitz, Aurel Perren, Günter Klöppel

Martin Anlauf, Nele Garbrecht, Tobias Henopp, Regina Schlenger, Günter Klöppel, Department of Pathology, University of Kiel, Germany
Anja Schmitt, Paul Komminoth, Philipp U Heitz, Aurel Perren, Department of Pathology, University of Zürich, Switzerland
Regina Schlenger, Department of Forensic Medicine, University of Kiel, Germany
Andreas Raffel, Markus Krausch, Claus F Eisenberger, Wolfram T Knoefel, Department of General, Visceral and Pediatric Surgery, University of Düsseldorf, Germany
Oliver Gimm, Henning Dralle, Department of General and Visceral Surgery, University of Halle-Wittenberg, Germany
Paul Komminoth, Department of Pathology, Kantonsspital Baden, Switzerland
Supported by the Hensel Stiftung Kiel (F370011, MA and GK), the Swiss National Foundation (SNF 31-61884, AP and PK) and the German Society of Pathology (MA). Tobias Henopp has a fellowship sponsored by Ipsen GMBH, Ettlingen, Germany
Correspondence to: Dr. Martin Anlauf, Department of Pathology, University of Kiel, Michaelisstr.11, 24105 Kiel, Germany. manlauf@path.uni-kiel.de
Telephone: +49-431-5971138 Fax: +49-431-5973462
Received: 2006-06-11 Accepted: 2006-07-07

Abstract

Gastrinomas are defined as gastrin secreting tumors that are associated with Zollinger-Ellison syndrome (ZES). ZES is characterized by elevated fasting gastrin serum levels, positive secretin stimulation test and clinical symptoms such as recurrent peptic ulcer disease, gastroesophageal reflux disease and occasional diarrhea. Genetically, nonhereditary (sporadic) gastrinomas are distinguished from hereditary gastrinomas, which are associated with multiple endocrine neoplasia type 1 (MEN1) syndrome. In general, duodenal gastrinomas are small and solitary if they are sporadic and multiple as well as hereditary. The sporadic gastrinomas occur in the duodenum or in the pancreas while the hereditary gastrinomas almost all occur in the duodenum. Our series of 77 sporadic duodenal neuroendocrine tumors (NETs) includes 18 patients (23.4%) with gastrinomas and ZES. Of 535 sporadic NETs in the pancreas collected from the NET archives of the departments of pathology in Zürich, Switzerland, and Kiel, Germany, 24 patients (4.5%) suffered from sporadic pancreatic gastrinomas and ZES. These NETs have to be distinguished from tumors with immunohistochemical positivity for gastrin but without evidence of ZES. An additional 19 patients suffered from MEN1 and ZES. These patients showed exclusively duodenal gastrinomas, but not pancreatic gastrinomas. The prognosis of sporadic and MEN1-associated duodenal gastrinomas is better than that of pancreatic gastrinomas, since they progress slowly to liver metastasis. In summary, sporadic and MEN1-associated gastrinomas in the duodenum and pancreas show different clinico-pathological and genetic features. The incidence of sporadic duodenal gastrin-producing tumors is increasing, possibly due to optimized diagnostic procedures. In contrast, pancreatic MEN1-associated gastrinomas seem to be extremely rare. A considerable subset of tumors with immunohistochemical expression of gastrin but without evidence of ZES should be designated as functionally inactive NETs expressing gastrin, but not as gastrinomas.

Key words: Endocrine tumor; Gastrinoma; Multiple endocrine neoplasia type 1; Precursor lesion; Zollinger-Ellison syndrome

Anlauf M, Garbrecht N, Henopp T, Schmitt A, Schlenger R, Raffel A, Krausch M, Gimm O, Eisenberger CF, Knoefel WT, Dralle H, Komminoth P, Heitz PU, Perren A, Klöppel G. Sporadic versus hereditary gastrinomas of the duodenum and pancreas: Distinct clinico-pathological and epidemiological features. *World J Gastroenterol* 2006; 12(34): 5440-5446

http://www.wjgnet.com/1007-9327/12/5440.asp

INTRODUCTION

Gastrinomas are defined as gastrin-producing tumors that are associated with Zollinger-Ellison syndrome (ZES) due to inappropriate gastrin secretion. ZES is characterized by elevated fasting gastrin serum levels, positive gastrin secretin stimulation test and clinical symptoms such as recurrent peptic ulcer disease, gastroesophageal reflux disease and occasional diarrhea[1,2].

The first cases of ZES were described in 1955[3].

One patient, a 36-year-old woman with severe recurrent ulcer disease and a family history strongly suggestive of multiple endocrine neoplasia type 1 (MEN1) background, was found to have several endocrine tumors, including microadenomas, in the pancreas. This case report already illustrates many of the issues that are still encountered in the diagnosis of gastrinoma. A closer look at the report by Zollinger and Ellison, on the basis of our current knowledge on gastrinomas, reveals that they were most likely dealing with a MEN1 patient. What is the reason for this assumption? Zollinger and Ellison described multiple endocrine tumors in the pancreas, which they thought were the cause of the ulcer syndrome, since their removal by a Whipple resection cured the patient. However, today we know that multiple gastrinomas virtually do not exist in the pancreas, but virtually always occur in the duodenum in the setting of MEN1. In this hereditary syndrome duodenal tumors producing gastrin are tiny and usually associated with multiple pancreatic tumors that do not produce gastrin, but may be large. In 1955, it was not possible to prove that the tumors produced gastrin. Firstly, gastrin still has to be isolated[4,5], and secondly, immunohistochemistry for gastrin has not yet been invented. Therefore, it is a quite likely assumption that Drs. Zollinger and Ellison's patient suffered from a recurrent ulcer disease in the setting of MEN1 syndrome and had multiple small gastrinomas in the duodenum, which were removed together with non-gastrin producing endocrine tumors in the pancreas. While the tumors in the pancreas were easily noticed and described, the duodenal minigastrinomas probably escaped detection.

This review focuses on the clinical setting and morphological aspects of sporadic and MEN1-associated duodenal and pancreatic gastrinomas. In addition, the results of an analysis of epidemiology of sporadic and MEN1-associated duodenal and pancreatic gastrinomas in a large series of duodenal and pancreatic neuroendocrine tumors (NETs) from the Swiss and German NET archives are presented.

CLINICAL SETTING OF GASTRINOMAS

Between 60% and 75% of patients with ZES are found to have an isolated duodenal or pancreatic gastrinoma (sporadic ZES). In the remaining patients ZES is part of MEN1 syndrome and these patients usually exhibit multiple duodenal gastrinomas (hereditary gastrinoma)[6-8]. The term pseudo-ZES (also called ZES type 1, as opposed to ZES type 2 caused by a gastrinoma) is coined for a syndrome with symptoms similar to ZES that appears to be caused by antral G-cell hyperfunction and hyperplasia[9,10]. The fact that this syndrome has no longer been described in recent years raises questions of whether it exists at all. In rare cases the syndrome of recurrent and intractable peptic ulceration may be found in association with a pancreatic endocrine tumor that does not produce and secrete gastrin[11]. The factor causing peptic ulceration in these patients has yet to be identified[12].

Among the gastroenteropancreatic neuroendocrine tumors associated with hormonal syndrome, gastrinomas are second only in incidence to insulinomas and are malignant in more than 60% of the cases. These tumors are classified as low grade malignant neoplasms, i.e. well differentiated neuroendocrine carcinomas. The peak incidence of gastrinomas lies between 40 and 50 years, children (5-15 years of age) are rarely affected[1].

SPORADIC GASTRINOMA

Sporadic gastrinomas occur either in the pancreas or in the duodenum and are apparently solitary tumors. In the past, approximately 70%-80% of these gastrinomas were thought to occur in the pancreas, particularly in its head. Currently, gastrinomas are more frequently found in the duodenum.

In general, gastrinomas represent the only type of endocrinologically active duodenal NETs, while all other types of duodenal NET (i.e. functionally inactive NETs expressing somatostatin, serotonin or gastrin, gangliocytic paraganglioma and poorly differentiated neuroendocrine carcinomas) are found to be endocrinologically silent. The reason for the increasing incidence of sporadic duodenal gastrinomas and endocrinologically silent inactive gastrin-producing NETs may be that many of these small NETs were overlooked but their large periduodenal/peripancreatic lymph node metastases were noted and recorded as primary gastrinoma in the pancreas or as primary lymph node gastrinoma in the past (details are shown below).

Sporadic duodenal gastrinomas usually arise from the first part of the duodenum and are located in the submucosa. They are most often less than 1 cm in diameter[13-16] (Figure 1A and 1B). Despite this small size metastases to regional lymph nodes are already found in 60% to 80% of the patients at the time of diagnosis[13]. It seems that periduodenal and peripancreatic lymph node metastases may grow faster than their duodenal primary tumors and thus may form large tumors that are easily recognized, in contrast to the duodenal primary tumors. It has therefore been suggested that the so-called peripancreatic and periduodenal lymph node gastrinomas that were described in the past may in fact be metastases of duodenal microgastrinomas that are overlooked during diagnostic work-up and surgery, rather than true primary tumors[17-19]. Apart from lymph node metastases, duodenal gastrinomas may metastasize to the liver, but only in a small percentage of cases (about 10%) and only many years after the manifestation of the disease[13]. Thus the10-year survival rate of 84% has been reported in patients with duodenal gastrinomas[20,21]. Fast growing and metastasizing duodenal gastrinomas are rare.

Histologically, duodenal gastrinomas are often submucosal tumors that infiltrate the mucosa and may also infiltrate the muscular layer if they are larger than 1 cm in diameter. They most often show a trabecular or pseudoglandular pattern. Their proliferative activity is usually between 2% and 10% (Figure 2). Some of the tumors may show angioinvasion. Their prognostic classification is outlined in detail in Table 1. Immunocytochemically, gastrin can be detected in all tumors[7,22]. Many duodenal gastrinomas are multihormonal and additionally contain single somatostatin or serotonin

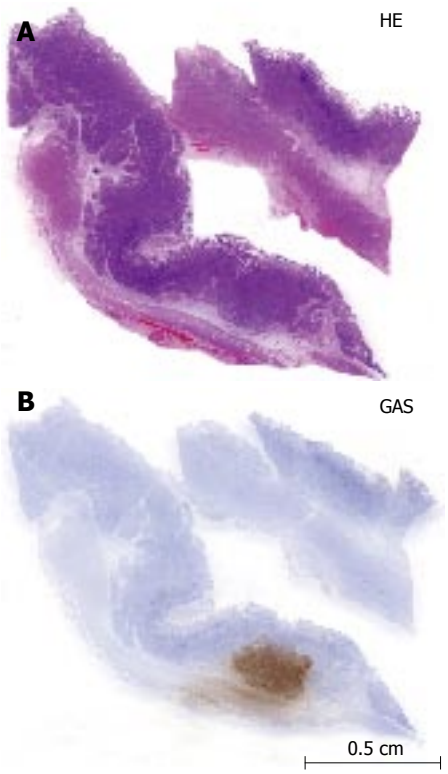


Figure 1 Overview of sporadic gastrinomas less than 3 mm in size within the submucosa stained with hematoxylin and eosin (**A**) and gastrin (**B**).

expressing cells in addition to gastrin cells.

Sporadic gastrinomas in the pancreas usually have a diameter of 2 cm or more (Figure 3). It has been reported that they occur more frequently in the head of the pancreas[13]. However, in our series they were found in all parts of the organ.

Metastasis of sporadic pancreatic gastrinomas to regional lymph nodes is found in approximately 60% of patients at the time of diagnosis[23], and liver metastases occur more frequently (10%-20%) than duodenal gastrinoma liver metastasis[17,18,23]. Thus the 10-year survival rate is worse in patients with pancreatic gastrinomas (57%) than in patients with duodenal gastrinomas (84%)[20,21]. In rare cases, bone metastases may develop in the terminal phase of a metastasized gastrinoma.

Histologically, pancreatic gastrinomas are similar to duodenal gastrinomas, but may have a higher proliferation and angioinvasion rate. Table 1 shows their prognostic assessment. Immunocytochemically, gastrin can be detected in almost all tumors[7,22]. Approximately 50% of gastrinomas are multihormonal and contain PP, glucagon and/or insulin in addition to gastrin.

Islet hyperplasia and nesidioblastosis have repeatedly been described in the non-neoplastic pancreas of patients with gastrinomas, but these findings cannot be confirmed by morphometry[24]. Recently, however, morphometrically defined PP-cell hyperplasia has been described in the ventrally derived region of the pancreatic head[25]. It has not been definitely established whether hypergastrinemia can influence these changes. In the stomach mucosa, however, sustained hypergastrinemia induces parietal cell hyperplasia with thickened mucosal folds and gastric acid hypersecretion. In addition, the number of enterochromaffin-like (ECL) cells is increased

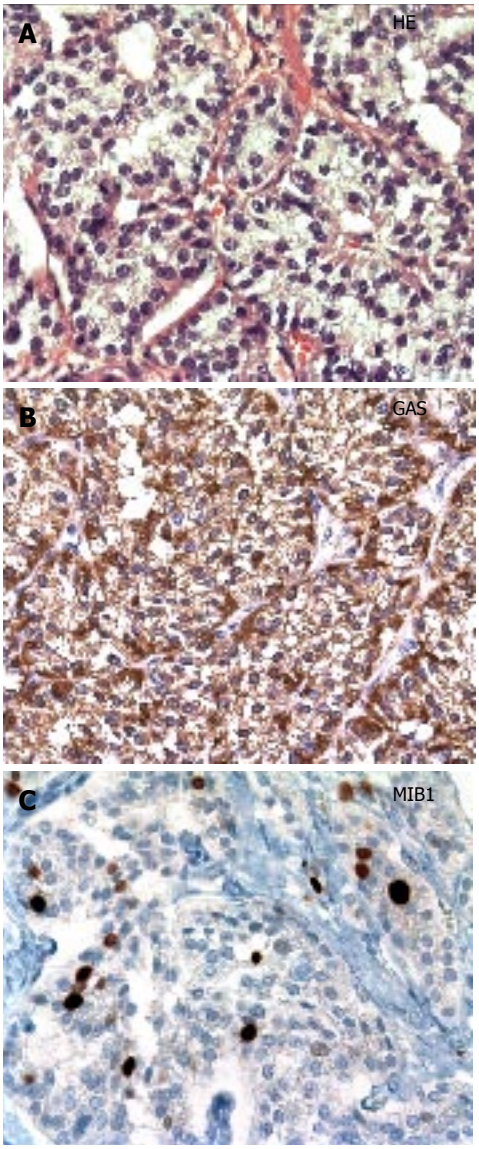


Figure 2 Morphology of sporadic duodenal gastrinoma stained with HE showing a trabecular and glandular growth pattern (**A**); strong immunoreactivity for gastrin (**B**), and expression of the nuclear proliferation antigen MIB1 in more than 2% of NET cells (**C**).

in the fundal mucosa[26-28]. ECL cell tumors in the fundus of the stomach, which are a well-known complication in patients suffering from pernicious anemia due to chronic type A gastritis, appear to be very uncommon in patients with sporadic ZES[7]. They have, however, been reported in patients with ZES and MEN1. In these instances they probably represent another neoplastic manifestation of MEN1 syndrome (see below) rather than merely the result of a trophic effect of gastrin[26,29].

MEN1-ASSOCIATED GASTRINOMAS

Approximately 25%-33% of patients with gastrinomas develop these tumors in the setting of MEN1. Almost all these gastrinomas reside in the duodenum[30]. They are usually smaller than 1 cm in diameter and multicentric, arising from multifocal hyperplastic gastrin cell proliferations[31] (Figures 4 and 5). Histologically,

Table 1 Classification of neuroendocrine tumors of the pancreas (WHO classification 2004)[41]

1 Well-differentiated neuroendocrine tumor
- Benign: confined to pancreas, < 2 cm in size, nonangioinvasive, ⩽ 2 mitoses/HPF and ⩽ 2% Ki-67-positive cells
  - Functioning: insulinoma
  - Nonfunctioning
- Benign or low grade malignant (uncertain malignant potential): confined to pancreas, ⩾ 2 cm in size, > 2 mitoses/HPF, > 2% Ki-67-positive cells, or angioinvasive
  - Functioning: gastrinoma, insulinoma, VIPoma, glucagonoma, somatostatinoma, or ectopic hormonal syndrome
  - Nonfunctioning

2 Well-differentiated neuroendocrine carcinoma
- Low grade malignant: invasion of adjacent organs and/or metastases
  - Functioning: gastrinoma, insulinoma, glucagonoma, VIPoma, somatostatinoma or ectopic hormonal syndrome
  - Nonfunctioning

3 Poorly-differentiated neuroendocrine carcinoma
- High grade malignant

Table 2 Classification of neuroendocrine tumors of the duodenum and upper jejunum

1 Well-differentiated neuroendocrine tumor
- Benign: nonfunctioning, confined to mucosa-submucosa, nonangioinvasive, ⩽ 1 cm in size
  - Gastrin-producing tumor (upper part of the duodenum)
  - Serotonin-producing tumor
  - Gangliocytic paraganglioma (any size and extension, periampullary)
- Benign or low grade malignant (uncertain malignant potential): confined to mucosa-submucosa, with or without angioinvasion, or > 1 cm in size
  - Functioning gastrin-producing tumor (gastrinoma), sporadic or MEN-1 associated
  - Nonfunctioning somatostatin-producing tumor (ampullary region) with or without neurofibromatosis type 1
  - Nonfunctioning serotonin-producing tumor

2 Well-differentiated neuroendocrine carcinoma
- Low grade malignant: invasion of the muscularis propria and beyond or metastases
  - Functioning gastrin-producing carcinoma (gastrinoma), sporadic or MEN-1 associated
  - Nonfunctioning somatostatin-producing carcinoma (ampullary region) with or without neurofibromatosis type 1
  - Nonfunctioning or functioning carcinoma (with carcinoid syndrome)
  - Malignant gangliocytic paraganglioma

3 Poorly-differentiated neuroendocrine carcinoma
- High grade malignant

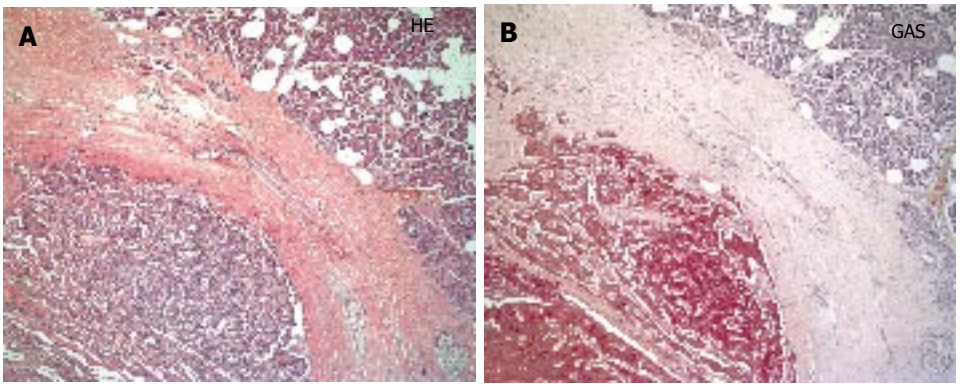


Figure 3 Overview of sporadic pancreatic gastrinoma surrounded by thickened collagen in the vicinity of normal pancreatic parenchyma stained with hematoxylin and eosin (**A**) and gastrin (**B**).

they show trabecular and pseudoglandular patterns and immunohistochemically they express gastrin and occasionally also somatostatin. Because of their small size they are (like sporadic duodenal gastrinomas) difficult to detect. Pancreatic gastrinomas associated with MEN1 are very rare[6,32], although the pancreas of these patients usually contains multiple endocrine micro- and macrotumors[33]. These tumors, however, virtually never produce significant amounts of gastrin[6,32]. The metastatic and biological behavior of duodenal MEN1-associated gastrinomas is similar to that of sporadic counterparts (Table 2).

EXTRADUODENAL AND EXTRAPANCREATIC GASTRINOMAS

Unusual sites of gastrinomas are the stomach[34], jejunum[35,36], biliary tract, liver[37] and kidney[38]. Ovarian or pancreatic mucinous cystic tumors that contain a sufficient number of active endocrine cells with gastrin production

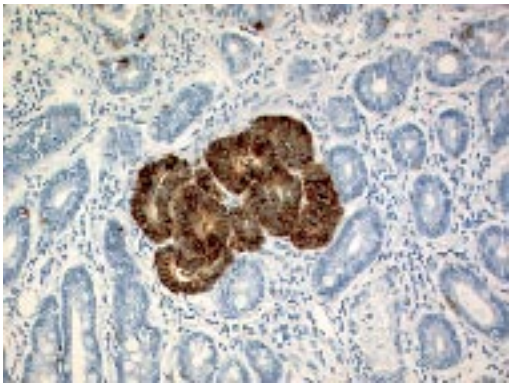


Figure 4 Circumscribed linear and nodular hyperplasia of gastrin cells within the Brunner's glands in a patient with MEN1.

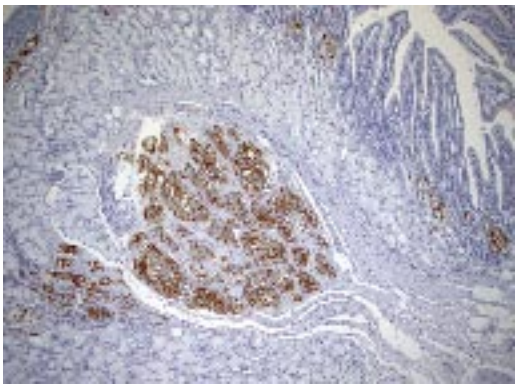


Figure 5 Tiny MEN1-associated duodenal gastrinoma within the submucosa revealing a diameter of less than 1 mm.

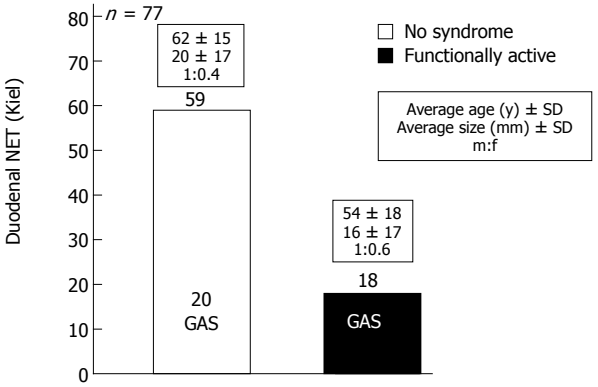


Figure 6 Duodenal NETs in the Kiel tumor archive. Fifty-nine (76.6%) out of the 77 NETs were endocrinologically not active, 20 of them expressed gastrin. These were not associated with ZES. All the functionally active NETs (18; 23.4%) were immunohistochemically positive for gastrin and showed a ZES.

may also cause ZES, but are uncommon[39-41].

PERSONAL OBSERVATIONS

In our series of sporadic duodenal NETs collected from 1975 to 2006, duodenal gastrin producing tumors account for 49.4% (38) of 77 sporadic NETs (Figure 6). Surprisingly, only 47.4% (18) of the gastrin-immunoreactive sporadic NETs show an association

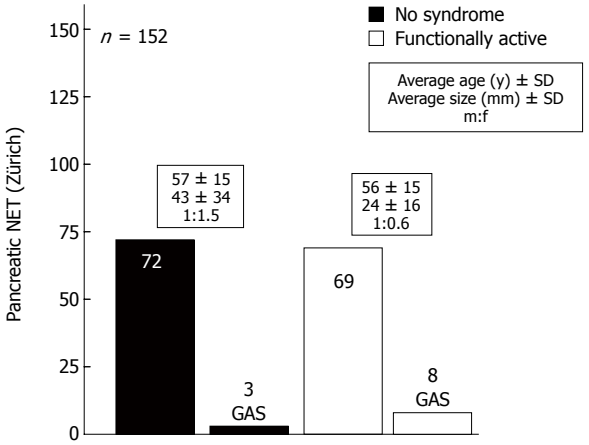


Figure 7 Pancreatic NETs in the Zürich tumor archives. Seventy-five (49.3%) out of the 152 pancreatic NETs were endocrinologically not active, of which 3 expressed gastrin. These 3 gastrin-expressing NETs were not associated with ZES. Eight of the 77 functionally active NETs were immunohistochemically positive for gastrin and associated with ZES.

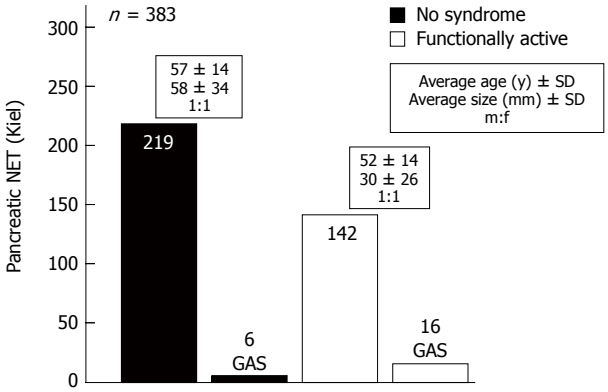


Figure 8 Pancreatic NETs in the Kiel tumor archives. Two hundred and twenty-five (58.7%) out of the 383 pancreatic NETs were endocrinologically not active. These gastrin-expressing NET were not associated with ZES. Sixteen of the 158 functionally active NETs were immunohistochemically positive for gastrin and showed a ZES.

with ZES (Figure 6). The reason for the lack of ZES in a considerable subset of patients with gastrin-expressing tumors remains to be analyzed in detail. Whether these gastrin producing tumors in the duodenum are similar in behavior to the duodenal gastrinomas remains unknown. However, it seems that they may have a different biology. Terminologically, these NETs with immunohistochemical expression of gastrin but without evidence of ZES should be designated as functionally inactive NETs expressing gastrin, but not as gastrinomas.

In two large series of sporadic pancreatic NETs from Kiel (n = 383) and Zürich (n = 152) pancreatic gastrinomas were found to be rare tumors, accounting for 4.2% (Kiel) and 5.3% (Zürich) of all collected sporadic tumors, respectively. Similar to duodenal tumors an additional 1.6 % (Kiel) and 2.0% (Zürich) of sporadic gastrin-expressing tumors were not associated with ZES and were therefore designated as functionally inactive pancreatic NETs producing gastrin (Figures 7 and 8).

It was reported that 19 (59.4%) of 32 patients with MEN1 showed ZES. The source of ZES in these patients

is duodenal rather than pancreatic gastrinomas. Most of these exhibit multifocal duodenal gastrinomas and lymph node metastases[31,33].

CONCLUSION

The preferred site of gastrinomas is the duodenum rather than the pancreas. Despite the small size of duodenal gastrinomas they may show the same rate of metastasis at the time of diagnosis as pancreatic gastrinomas, which are usually larger in size. However, the survival rate of patients with pancreatic gastrinomas is lower than that of patients with duodenal gastrinomas. MEN1- associated gastrinomas are virtually all localized in the duodenum. They are usually multiple. They probably arise from multifocal precursor lesions, i.e. diffuse gastrin cell proliferations that are lacking in sporadic duodenal gastrinomas. Biologically, the behavior of MEN1-associated gastrinomas is similar to that of sporadic duodenal gastrinomas. Gastrin expressing tumors both in the duodenum and in the pancreas without evidence of ZES should be designated as functionally inactive NETs producing gastrin, but not as gastrinomas. The reasons for the lack of hormonal symptoms in gastrin expressing NETs still need to be analyzed in detail.

ACKNOWLEDGMENTS

Some of the epidemiological data in this manuscript are part of Nele Garbrechts's and Regina Schlenger's MD theses.

REFERENCES

1 **Jensen RT**, Gardner JD. Gastrinoma. In: Go VLW, DiMagno EP, Gardner JD, Lebenthal E, Reber HA, Scheele GA. The pancreas: biology, pathobiology and disease. 2 ed. New York: Raven Press, 1993: 931-978
2 **Zollinger RM**. Gastrinoma: the Zollinger-Ellison syndrome. *Semin Oncol* 1987; **14**: 247-252
3 **Zollinger RM**, Ellison EH. Primary peptic ulcerations of the jejunum associated with islet cell tumors of the pancreas. *Ann Surg* 1955; **142**: 709-23; discussion, 724-8
4 **Gregory RA**, Tracy HJ. The preparation and properties of gastrin. *J Physiol* 1961; **156**: 523-543
5 **Gregory RA**, Tracy HJ, French JM, Sircus W. Extraction of a gastrin-like substance from a pancreatic tumour in a case of Zollinger-Ellison syndrome. *Lancet* 1960; **1**: 1045-1048
6 **Klöppel G,** Willemer S, Stamm B, Häcki WH, Heitz PU. Pancreatic lesions and hormonal profile of pancreatic tumors in multiple endocrine neoplasia type I. An immunocytochemical study of nine patients. *Cancer* 1986; **57**: 1824-1832
7 **Solcia E**, Capella C, Klöppel G. Tumors of the pancreas. AFIP Atlas of Tumor Pathology, third series, fascicle 20. Washington, DC: Armed Forces Institute of Pathology, 1997
8 **Ruszniewski P**, Podevin P, Cadiot G, Marmuse JP, Mignon M, Vissuzaine C, Bonfils S, Lehy T. Clinical, anatomical, and evolutive features of patients with the Zollinger-Ellison syndrome combined with type I multiple endocrine neoplasia. *Pancreas* 1993; **8**: 295-304
9 **Polak JM**, Stagg B, Pearse AG. Two types of Zollinger-Ellison syndrome: immunofluorescent, cytochemical and ultrastructural studies of the antral and pancreatic gastrin cells in different clinical states. *Gut* 1972; **13**: 501-512
10 **Friesen SR**, Tomita T. Pseudo-Zollinger-Ellison syndrome: hypergastrinemia, hyperchlorhydria without tumor. *Ann Surg* 1981; **194**: 481-493
11 **Mehring UM,** Jäger HJ, Klöppel G, Hasse FM. [Pancreatic polypeptide secreting endocrine pancreas tumor associated with multiple stomach and duodenal ulcers]. *Langenbecks Arch Chir* 1997; **382**: 134-137
12 **Chey WY**, Chang TM, Lee KY, Sun G, Kim CK, You CH, Hamilton DL, Shah A, Rhee JC, Mutt V. Ulcerogenic tumor syndrome of the pancreas associated with a nongastrin acid secretagogue. *Ann Surg* 1989; **210**: 139-149
13 **Donow C**, Pipeleers-Marichal M, Schröder S, Stamm B, Heitz PU, Klöppel G. Surgical pathology of gastrinoma. Site, size, multicentricity, association with multiple endocrine neoplasia type 1, and malignancy. *Cancer* 1991; **68**: 1329-1334
14 **Oberhelman HA Jr**, Nelsen TS. Surgical consideration in the management of ulcerogenic tumors of the pancreas and duodenum. *Am J Surg* 1964; **108**: 132-141
15 **Stamm B**, Hedinger CE, Saremaslani P. Duodenal and ampullary carcinoid tumors. A report of 12 cases with pathological characteristics, polypeptide content and relation to the MEN I syndrome and von Recklinghausen's disease (neurofibromatosis). *Virchows Arch A Pathol Anat Histopathol* 1986; **408**: 475-489
16 **Thompson NW**, Vinik AI, Eckhauser FE. Microgastrinomas of the duodenum. A cause of failed operations for the Zollinger-Ellison syndrome. *Ann Surg* 1989; **209**: 396-404
17 **Pipeleers-Marichal M**, Donow C, Heitz PU, Klöppel G. Pathologic aspects of gastrinomas in patients with Zollinger-Ellison syndrome with and without multiple endocrine neoplasia type I. *World J Surg* 1993; **17**: 481-488
18 **Delcore R Jr**, Cheung LY, Friesen SR. Outcome of lymph node involvement in patients with the Zollinger-Ellison syndrome. *Ann Surg* 1988; **208**: 291-298
19 **Wolfe MM**, Alexander RW, McGuigan JE. Extrapancreatic, extraintestinal gastrinoma: effective treatment by surgery. *N Engl J Med* 1982; **306**: 1533-1536
20 **Weber HC**, Venzon DJ, Lin JT, Fishbein VA, Orbuch M, Strader DB, Gibril F, Metz DC, Fraker DL, Norton JA. Determinants of metastatic rate and survival in patients with Zollinger-Ellison syndrome: a prospective long-term study. *Gastroenterology* 1995; **108**: 1637-1649
21 **Thompson JC**, Lewis BG, Wiener I, Townsend CM Jr. The role of surgery in the Zollinger-Ellison syndrome. *Ann Surg* 1983; **197**: 594-607
22 **Heitz PU**, Kasper M, Polak JM, Klöppel G. Pancreatic endocrine tumors. *Hum Pathol* 1982; **13**: 263-271
23 **Stabile BE**, Passaro E Jr. Benign and malignant gastrinoma. *Am J Surg* 1985; **149**: 144-150
24 **Schwarting H**, Osse G, Sippel M, Arnold R, Creutzfeldt W. Morphometry of the pancreatic islets in patients with insulinomas and gastrinomas. In: Mutt V, Uvnäs-Moberg K. Regulatory peptides. Abstracts of the 4th international symposium on gastrointestinal hormones. Amsterdam: Elsevier, 1983: 129
25 **Martella EM**, Ferraro G, Azzoni C, Marignani M, Bordi C. Pancreatic-polypeptide cell hyperplasia associated with pancreatic or duodenal gastrinomas. *Hum Pathol* 1997; **28**: 149-153
26 **Bordi C**, Costa A, Missale G. Letter: ECL cell proliferation and gastrin levels. *Gastroenterology* 1975; **68**: 205-206
27 **Solcia E**, Bordi C, Creutzfeldt W, Dayal Y, Dayan AD, Falkmer S, Grimelius L, Havu N. Histopathological classification of nonantral gastric endocrine growths in man. *Digestion* 1988; **41**: 185-200
28 **D'Adda T**, Corleto V, Pilato FP, Baggi MT, Robutti F, Delle Fave G, Bordi C. Quantitative ultrastructure of endocrine cells of oxyntic mucosa in Zollinger-Ellison syndrome. Correspondence with light microscopic findings. *Gastroenterology* 1990; **99**: 17-26
29 **Solcia E**, Capella C, Fiocca R, Rindi G, Rosai J. Gastric argyrophil carcinoidosis in patients with Zollinger-Ellison syndrome due to type 1 multiple endocrine neoplasia. A newly recognized association. *Am J Surg Pathol* 1990; **14**: 503-513
30 **Pipeleers-Marichal M**, Somers G, Willems G, Foulis A, Imrie C, Bishop AE, Polak JM, Häcki WH, Stamm B, Heitz PU.

Gastrinomas in the duodenums of patients with multiple endocrine neoplasia type 1 and the Zollinger-Ellison syndrome. *N Engl J Med* 1990; **322**: 723-727

31 **Anlauf M**, Perren A, Meyer CL, Schmid S, Saremaslani P, Kruse ML, Weihe E, Komminoth P, Heitz PU, Klöppel G. Precursor lesions in patients with multiple endocrine neoplasia type 1-associated duodenal gastrinomas. *Gastroenterology* 2005; **128**: 1187-1198

32 **Vella MA**, Cowie AG, Gorsuch AN, Watson LC. Giant gastrinoma in a patient with multiple endocrine adenopathy (type 1). *J R Soc Med* 1988; **81**: 359-360

33 **Anlauf M**, Schlenger R, Perren A, Bauersfeld J, Koch CA, Dralle H, Raffel A, Knoefel WT, Weihe E, Ruszniewski P, Couvelard A, Komminoth P, Heitz PU, Klöppel G. Microadenomatosis of the endocrine pancreas in patients with and without the multiple endocrine neoplasia type 1 syndrome. *Am J Surg Pathol* 2006; **30**: 560-574

34 **Klöppel G**, Clemens A. The biological relevance of gastric neuroendocrine tumors. *Yale J Biol Med* 1996; **69**: 69-74

35 **Solcia E**, Capella C, Buffa R, Grigerio G, Riocca R. Pathology of the Zollinger-Ellison syndrome. In: Fenoglio LM, Wolf M. Progress in surgical pathology, vol. 1. New York: Masson, 1980: 119-133

36 **Antonioli DA**, Dayal Y, Dvorak AM, Banks PA. Zollinger-Ellison syndrome. Cure by surgical resection of a jejunal gastrinoma containing growth hormone releasing factor. *Gastroenterology* 1987; **92**: 814-823

37 **Stabile BE**, Morrow DJ, Passaro E Jr. The gastrinoma triangle: operative implications. *Am J Surg* 1984; **147**: 25-31

38 **Nord KS**, Joshi V, Hanna M, Khademi M, Saad S, Marquis J, Pelzman H, Verner E. Zollinger-Ellison syndrome associated with a renal gastrinoma in a child. *J Pediatr Gastroenterol Nutr* 1986; **5**: 980-986

39 **Margolis RM**, Jang N. Zollinger-Ellison syndrome associated with pancreatic cystadenocarcinoma. *N Engl J Med* 1984; **311**: 1380-1381

40 **Morgan DR**, Wells M, MacDonald RC, Johnston D. Zollinger-Ellison syndrome due to a gastrin secreting ovarian mucinous cystadenoma. Case report. *Br J Obstet Gynaecol* 1985; **92**: 867-869

41 **Heitz Ph U**, Komminoth P, Perren A, Klimstra DS, Dayal Y, Bordi C, Lechagon J, Centeno BA, Klöppel G. Tumors of the endocrine pancreas. In: DeLellis RA, Lloyd RV, Heitz PU, and Eng C, eds. Pathology and genetics: tumours of endocrine organs. WHO classification of tumors. Lyon: IARC Press, 2004: 175-208

S- Editor Wang J **L- Editor** Wang XL **E- Editor** Liu WF

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5447-5457
World Journal of Gastroenterology ISSN 1007-9327

REVIEW

Do probiotics have a therapeutic role in gastroenterology?

Jimmy K Limdi, Catherine O'Neill, John McLaughlin

Jimmy K Limdi, Fairfield Hospital Bury, Lancashire, United Kingdom
Catherine O'Neill, Hope Hospital, Manchester, United Kingdom
John McLaughlin, Consultant Gastroenterologist, Lecturer in GI Sciences, Hope Hospital, Manchester, United Kingdom
Correspondence to: Dr. Jimmy K Limdi, Specialist Registrar in Gastroenterology, Fairfield HospitalBury, Lancashire, United Kingdom. jklimdi@doctors.org.uk
Telephone: +44-161-7646081
Received: 2006-04-02 Accepted: 2006-06-14

Abstract

Several hundred species of bacteria inhabit the gut, and affect its cell biology, morphology and homeostasis. Many bacteria are however potential pathogens, especially if the integrity of the epithelial barrier is physically or functionally breached. Conversely, the interaction between host and commensal microbes can confer important health benefits. This has led to commercial and public interest in 'probiotics', live microbes principally taken as food supplements. Might probiotics also be used in disease therapy? Experimental evidence that probiotics modulate gut physiology, particularly barrier integrity and immunological function, underpins exciting new gastroenterological research. We discuss below the scientific basis for probiotic effects and present a critical perspective for their use in relation to gastrointestinal disease.

Key words: Probiotics; Gastroenterology

Limdi JK, O'Neill C, McLaughlin J. Do probiotics have a therapeutic role in gastroenterology? *World J Gastroenterol* 2006; 12(34): 5447-5457

http://www.wjgnet.com/1007-9327/12/5447.asp

INTRODUCTION

The concept of probiotics probably dates back to 1908, when Nobel Prize winner Eli Metchnikoff suggested that the long life of Bulgarian peasants resulted from their consumption of fermented milk products[1]. In 1965 Lilly and Stillwell first used the term 'probiotic' when describing 'substances secreted by one organism which stimulate the growth of another'[2]. Parker[3] described probiotics as 'organisms and substances which contribute to intestinal microbial balance' and Fuller proposed in 1989 that probiotics were 'a live microbial supplement which beneficially affects the host animal by improving its microbial balance[4]. Salminen *et al*[5] defined them as 'foods containing live bacteria which are beneficial to health', whilst Marteau *et al*[6] define them as 'microbial preparations or components of microbial cells that have a beneficial effect to health and well being'. Such definitions underpin the current popular commercial usage of various 'friendly bacteria' to secure non-specific benefits to health.

With improved understanding of the physiology and therapeutic role of probiotics, definitions have evolved bolder claims, which now enter medical territory. Charteris *et al*[7] defined probiotics as 'micro-organisms, which when ingested, may have a positive effect in the prevention and treatment of a specific pathological condition'.

Two related terms are prebiotics and synbiotics: *prebiotics* are defined as "non digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health"[8]. When prebiotics and probiotics are administered together, this is referred to as a synbiotic.

NORMAL GUT MICROFLORA

The colon contains tenfold more bacteria than the total number of mammalian cells constituting the host. The relationship is symbiotic: for example, bacterial vitamin K synthesis contributes to haemostasis, whilst short chain fatty acids generated by colonic bacteria salvage additional energy from otherwise 'wasted' dietary fibre.

Colonisation of the gastrointestinal tract starts immediately after birth, initially with maternal vaginal and intestinal flora. Other sources are diet (breast or formula based feeds)[9-12] and environment, as reflected by the different gut flora in infants born in developing and developed countries[13-15]. Infants who are breast fed predominantly harbour *Bifidobacteria* whilst formula fed infants have a more complex bacterial profile comprising *Enterobacteria*, *Bacteroides*, *Clostridia*, *Lactobacilli*, *Bifidobacteria* and *Streptococci*[11].

These 'pioneer' bacteria are important because they modulate epithelial cell gene expression, creating a favourable habitat for themselves by inhibiting the growth of bacteria introduced later[16]. This renders initial colonisation causally determinant to the final composition of bacterial flora in adults[17].

During development, the gut flora changes. The mouth

harbours mainly facultative and strict anaerobes including *Streptococci*, *Bacteroides* and yeasts. The oesophagus has no significant resident microbial colonisation but is constantly transited by swallowed organisms. The widely held idea that the upper gut is largely sterile is not valid. The stomach and duodenum harbour up to 10^4 colony forming units (CFU) per gm of *Candida albicans, Bacteroides*, *Lactobacillus* and *Streptococcus*. *H pylori* are specifically adapted for gastric residence. The jejunum again harbours *Bacteroides*, Candida albicans, *Lactobacillus* and *Streptococcus* but with a content of 10^5-10^7 CFU/g. Scepticism that probiotics will not survive the passage through the acidic stomach is therefore unfounded. From the ileum onwards bacterial colonisation increases from 10^7-10^8 CFU/g in the ileum to 10^{10}-10^{11} CFU/g in the colon, with a predominance of *Bacteroides*, Bacillus, Clostridium, Enterococcus, *Peptostreptococcus*, and *Streptococcus* species[18].

GUT MICROFLORA AND GUT PHYSIOLOGY

Recent research has demonstrated that a dynamic and reciprocal interplay exists between the gut microflora and the host. In particular, there is growing evidence that bacteria play an important role in directing epithelial differentiation and reinforcement of the gut barrier, through complex host-bacterial cross talk which occurs at a molecular level in interacting cells. This new biology underpins the putative effects of probiotics, since host-bacterial interactions can be re-engineered by purposeful manipulation of luminal ecology.

The physical barrier: bacteria and tight junctions

The intestinal epithelium constitutes an anatomical and functional barrier, effectively a bipolar monocellular obstacle between luminal microbes and the cells of the lamina propria. Barrier function is normally maintained by a complex interplay of numerous proteins, assembled to form the tight junction complexes (TJs)[19,20] in the juxtaapical region of the cell membrane (Figure 1). Commensal organisms contribute uncharacterised constitutive signals supporting epithelial integrity. Disruption of TJs may be elicited by pathogenic bacteria, stress and injury, *via* pro-inflammatory cytokines. Disruption of TJ integrity results in increased paracellular permeability, which is measurable in a variety of experimental models, and which may initiate, exacerbate or perpetuate intestinal inflammation in disease[21]. Altered intestinal permeability has been demonstrated in Crohn's disease, celiac disease, intestinal infections and NSAID-induced enteropathy[22]. In order to counteract the harmful effects of luminal pathogens and toxins, and to protect barrier homeostasis, intestinal epithelial cells exhibit several additional defensive features, which include production of defence peptides and mucins[23]. In addition, a class of bacterial-sensing immunocytes, the dendritic cells, are able to project sensory dendrites into the lumen between adjacent enterocytes *via* TJ regions. Dendritic cells express receptors evolved to sense bacterial components, through which highly patterned host immune responses are evoked appropriately and constantly. Supporting experiments in rats have shown

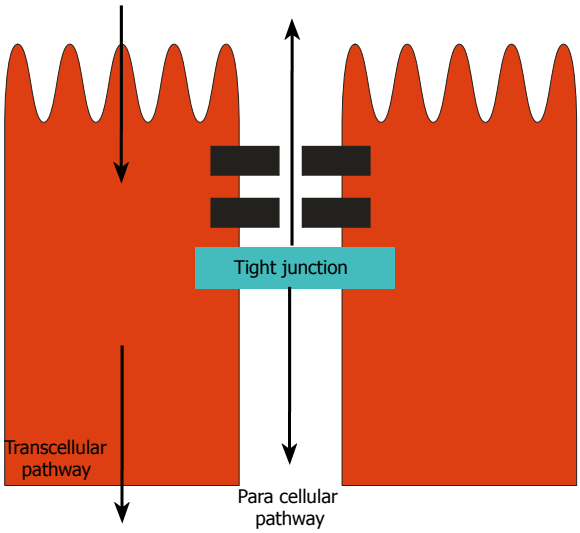


Figure 1 Tight junctions have a 'barrier' function in epithelia.

that colonisation of an excluded colonic loop with *E. coli* increased paracellular permeability, but this was partially reversed by colonisation with a putative probiotic, *Lactobacillus* brevis[24]. In addition, the chronic colitis occurring in Interleukin-10 (IL-10) deficient mice[25] is associated with increased colonic permeability, which is reversed in mice pre-treated for 4 wk. with a probiotic product, VSL#3 consisting of Bifidobacterium, *Streptococcus* and *Lactobacillus* species.

The potential cellular basis has also been addressed in human cell models[26]. The influence of whole probiotic bacteria *E. coli* Nissle 1917 (EcN) or VSL#3, bacterial cell lysates or conditioned medium from bacterial cultures were assessed against a variety of barrier and defensive parameters in human intestinal epithelial cell lines. These included a measure of TJ function (transepithelial resistance, TER) and tight junction protein abundance, IL-8 secretion and mucin gene expression. In addition, protective effects on pathogen (*Salmonella dublin*) induced alterations were analyzed. The probiotic mixture and soluble protein released from it, increased basal TER, prevented pathogen-induced decrease in TER, and stabilized TJs. This suggested that the organisms, or their secretory products, functionally modulate the intestinal epithelium of the host. These include competition of organisms for contact with the epithelial surface, stabilization of the cytoskeletal and barrier function, and the induction of mucin gene expression. Gram-negative and gram-positive organisms differed in the cellular mechanisms activated: perhaps a combination of organisms might be more effective than the application of a single strain[26]. The stumbling block inevitably lies in translating observations in these reductionist models to a proven clinical effect.

Recent interest has centred on regulation of the barrier *via* epithelial sensing of microbial components. The Toll-like receptors (TLRs) are a class of pattern-recognition receptors that specifically discriminate between self and microbial non-self based on the recognition of molecular patterns[27]. TLRs thereby play an important role in im-

mune responses and the induction of antimicrobial effector pathways, leading to elimination and exclusion of host-threatening pathogens. It has been shown that the intestinal epithelium expresses several TLRs, which include TLR2, TLR3, TLR4 and TLR5[24-34]. It is possible that certain probiotic agents contain TLR-specific immunostimulatory features, leading to the amelioration of colitis by restoring intestinal epithelial barrier to protect the host[35].

The functional barrier: probiotics and mucosal immunity

The gastrointestinal mucosa is the primary interface between the external environment and the immune system. In the complete absence of intestinal microflora antigen transport is increased, indicating that the normal gut microflora maintain gut defences[25]. The microflora affect the development of gut associated lymphoid tissue at an early age by directing the regulation of systemic and local immune responsiveness, including promoting tolerance via hypo-responsiveness to antigens from micro-organisms and food[36]. Probiotic organisms have been shown to modulate immunoglobulin production. Secretory IgA plays an important role in mucosal immunity, contributing functionally to the barrier against pathogenic bacteria and viruses[37-40]. An enhanced IgA immune response has been shown in children with Crohn's disease treated with *Lactobacillus* GG[41]. Interestingly, Madsen *et al*[42] demonstrated that IL10 deficient mice displayed significantly higher basal numbers of adherent bacteria compared with healthy control mice. When the colon was repopulated with *Lactobacillus* reuteri enemas the proportion of adherent and translocated bacteria, and the development of colitis, was significantly decreased. Further, Schultz[43] demonstrated that feeding *Lactobacillus plantarum* attenuated established colitis in IL-10 knockout mice. In rats, the effects of *Lactobacilli* and fibre (oatbase) were studied in Methotrexate-induced enterocolitis. Rats received an intragastric infusion of an elemental diet, with or without supplementation of oatbase, *Lactobacillus reuteri* R2LC and *Lactobacillus plantarum* DSM9843[44]. Methotrexate was injected intraperitoneally on d 3. By d 6 *Lactobacillus* decreased intestinal inflammation, re-established intestinal microecology and reduced bacterial translocation to extra-intestinal sites.

Data from human studies support a role for the gut microflora in the development of several gut associated inflammatory conditions, most likely triggered by immune response to their antigenic structures[45]. Thus probiotics may exert clinical effects by altering the intestinal inflammatory response to the luminal microflora.

Trophic and nutritional effects of gut microflora

Probiotic organisms exert a potentially positive effect on gut function through a trophic action on gut mucosa. In experimental models, crypt cell turnover is reduced in the colon of rats bred in germ free environments (gnotobiotic animals). Germ free crypts also contain fewer cells than those of colonised rats[46]. Butyrate is an important source of energy for colonocytes[47], whilst acetate and propionate are found in portal blood and are eventually metabolised in the liver or peripheral tissues, in particular muscle[47,48]. The most important role of SCFA is probably their trophic effect on colonic epithelium. Short chain fatty acids stimulate epithelial cell proliferation and differentiation in large and small bowel *in vivo*[49]. Butyrate however inhibits cell proliferation in epithelial cell lines of neoplastic origin[50]. Further, butyrate is pro-apoptotic and promotes reversion of cells from neoplastic to non-neoplastic phenotypes[51]. SCFA generation can clearly be altered by manipulating colonic micro-ecology, so probiotics and prebiotics may find a role in the prevention of colonic neoplasia and in the therapy of inflammatory bowel diseases.

Colonic micro-organisms play an important role in vitamin synthesis[52,53] and in the absorption of calcium, magnesium and iron[54-56]. Ion absorption in the caecum is improved by carbohydrate, and production of short chain fatty acids particularly acetate, propionate, and butyrate.

Interactions with mucus

A mucus gel covers the gut epithelium acting as a protective barrier against pathogens and reducing physical trauma. Change in the mucus content or structure compromise barrier function. Interactions occur between bacteria and mucus, including the probiotic bacteria that bind to intestinal mucus[57]. This is potentially advantageous since it inhibits adhesion of enteropathogenic bacteria to mucus. For example *Enterococcus faecium* inhibits the adhesion of enterotoxigenic *E. coli* K88 to porcine small intestine mucus[58].

PROBIOTICS IN CLINICAL GASTROENTEROLOGY

The experimental data discussed above demonstrate that several 'probiotic' organisms exert biological effects which might translate into clinical benefits. Current clinical evidence is limited and non-uniform. Key observations in relevant conditions are discussed below.

Probiotics in GI infections

Probiotics have an emerging role in the treatment of gastrointestinal infections. Probably the best described is in acute infantile diarrhoea. *Lactobacillus* strain GG in fermented milk or freeze- dried powder was shown to reduce the duration of diarrhoea in acute rotavirus infection compared to a placebo group given pasteurised yoghurt[59]. Other studies have confirmed these results[60,61]. Suggested mechanisms of action are stabilisation of indigenous microflora[62], reduction in the increased gut permeability caused by rotavirus infection[63] and reduction in the duration of virus shedding[64]. This may be cause-specific. In a multicentre European trial of probiotics in acute childhood diarrhoea caused by rotavirus and other pathogens[65], probiotics (*Lactobacillus* GG) shortened the duration of diarrhoea in rotavirus diarrhoea, but showed no such effect with other pathogens.

Probiotics may also have a role in the prevention of acute infantile diarrhoea. In a double blind, placebo controlled trial hospitalised infants were randomised to receive a standard infant formula alone or supplemented with *Bifidobacterium bifidum* and *Streptococcus thermophilus*. After a 17-mo follow up period, 7% of those receiving a probiotic

had experienced diarrhoea compared to 31% given the standard formula[66]. Viral shedding was lower in the probiotic supplemented group. Prophylactic use of *Lactobacillus* GG in 204 undernourished children followed up for 15 mo also decreased the incidence of acute diarrhoea[67]. This effect was confined to non-breast fed infants. In a more recently reported double blind randomised placebo controlled trial, 81 children between 1-3 years of age, hospitalised for reasons other than diarrhoea, and were given *Lactobacillus* GG or placebo for the duration of their admission. The incidence of nosocomial diarrhoea was lower in the probiotic group (7% as compared to placebo, 33%)[68]. Furthermore, although the prevalence of rotavirus infection was similar in both groups, the risk of rotavirus gastroenteritis was lower in the probiotic group. Finally, the benefits of *Lactobacillus* GG in rotavirus associated diarrhoea mainly comprise a reduction in duration of diarrhoea by 1-2 d compared to a median of 3 d.

The positive results for *Lactobacillus* GG in acute viral diarrhoea cannot necessarily be extrapolated to other probiotic strains, nor to other causes of acute diarrhoea. The data imply species and disease specificity, For example, in a study comparing various probiotic strains, *Lactobacillus* GG was found to have a beneficial effect in rotavirus gastroenteritis but this was not shared by *L. rhamnosus*, *L. delbrueckii* or *Streptococcus thermophilus*[60]. *Saccharomyces boulardii* has also displayed beneficial effects in acute diarrhoea in children and adults[60,70]. Enterococcus SF68 in adults with acute diarrhoea has however shown inconsistent results[71-73]. It is unclear whether the modest benefits suggested by these studies justify routine use of probiotics in diarrheal illnesses since most acute diarrhoeal diseases are self limited. The benefit may be greatest in situations where patients are at risk for complications such as in children with malnutrition in developing countries.

Antibiotic-associated diarrhoea

Diarrhoea can occur as an adverse effect of antibiotic therapy, in up to 39% of antibiotic treated hospitalised patients[74]. Broad spectrum antibiotics are more commonly implicated, possibly because of more profound alteration in colonic flora[75]. Several placebo-controlled studies have shown a decrease in the incidence of diarrhoea or change in stool consistency when patients were treated with probiotics in addition to antibiotics[76-82]. The probiotic organisms studied were *Lactobacillus* spp., Enterococcus and *Saccharomyces boulardii*. Not all studies support this possibility. A recent study of 267 patients on antibiotics randomised to *Lactobacillus* GG or placebo failed to show any decrease in incidence of diarrhoea, with 29% in both groups developing symptoms[83]. Three other smaller studies were also negative[78,84,85]. Although most studies looking at probiotics in antibiotic-associated diarrhoea are placebo controlled and conducted on a reasonable number of subjects, different antibiotics were used in these studies contributing to their heterogeneity. Two recent meta-analyses (of nine and seven randomised placebo controlled double blind trials) have looked at the effect of probiotics in prevention of antibiotic-associated diarrhoea[86,87]. The meta- analysis by D'Souza *et al*[87]reviewed nine randomised, double blind, placebo controlled trials. Four trials used yeast (*Saccharomyces boulardii*); four used *Lactobacilli* and another used a strain of enterococcus that produced lactic acid. Three trials used a combination of probiotic bacteria. Antibiotics were given with probiotics (or with placebo, in the control group) in all nine trials. The combined odds ratio in favour of active treatment over placebo in preventing diarrhoea associated with antibiotics was 0.37 (95% confidence interval, 0.26 to 0.53; $P < 0.001$). In the meta- analysis by Cremonini *et al*[86], twenty two studies using *Lactobacillus* and Saccharomyces species, matched the inclusion criteria of which seven studies were homogenous. The combined relative risk was 0.399 (95% confidence interval, 0.27-0.57) suggesting a strong benefit of probiotic administration on antibiotic associated diarrhoea. The pooled results suggested that probiotic administration had an overall benefit. However, the published data is discordant in that it is unclear what the optimal dose and timing of supplementation should be.

Clostridium difficile associated diarrhoea (CDAD)

Clostridium difficile is a Gram positive bacterium that can cause colitis, mediated by two enterotoxins, enterotoxin A and B. The pathophysiology is not fully clear but risk factors include intercurrent or continued antibiotic therapy, elderly age, renal disease and female sex[88,89]. Probiotics have been partially evaluated in the prevention of CDAD. Early uncontrolled trials using *Lactobacillus* GG[90-92] and a preliminary report of a controlled trial using *Lactobacillus* GG suggested benefit in recurrent CDAD[93]. Similarly uncontrolled or open label studies[94,95] and subsequently two controlled trials[96,97] have suggested efficacy of *Saccharomyces boulardii* in recurrent CDAD. In the study by McFarland *et al*[94], 124 patients were studied, including 64 patients with an initial episode of CDAD and 60 who had at least one previous episode of CDAD. Subjects received oral *Saccharomyces boulardii* (1 g/d for 4 wk or placebo) and were followed up for an additional 4 wk after therapy. Multivariate analysis showed that patients treated with *S. boulardii* and standard antibiotics had a significantly lower risk of CDAD (relative risk 0.43; 95% confidence interval, 0.20-0.97) compared with placebo. In their subsequent study, Surawicz *et al*[97] tested patients receiving a standard antibiotic for 10 d and then added *S. boulardii* (1 g/d for 28 d) or placebo. A significant decrease in recurrence of CDAD (16.7%) was observed in patients treated with high-dose Vancomycin (2 g/d) compared with those receiving Vancomycin and placebo (50%; $P < 0.05$). Most studies were small and were mostly not placebo controlled.

A recent systematic review looking at studies in which probiotic therapy was used for prevention and treatment of *C. difficile*- associated diarrhoea concluded that the evidence for routine clinical use of probiotics in this setting was insufficient[98]. Again, although probiotics are generally considered safe, case reports have described *Saccharomyces cerevisiae* fungaemia and deaths in immunocompromised and critically ill patients who received a commercial preparation of *S. boulardii* (genomically identical to *S. cerevisiae*)[99]. Their routine use can therefore not be recommended.

Probiotics in Inflammatory Bowel Diseases

Although the etiology of inflammatory bowel diseases is

not entirely clear, dysfunctions in both innate and acquired immunity are implicated. There has been an increased interest in pathogenic and endogenous intestinal flora, with supportive data derived from several animal models. As noted above spontaneous colitis may develop in mice deficient in the immunoregulatory cytokine IL-10 but IL-10 deficient germ free mice remain disease free[100-102].

Clinical studies suggest a significant role for bacteria in the pathogenesis of human IBD. Crohn's disease activity has been shown to improve with antimicrobial therapy, faecal diversion[103,104], bowel rest and intestinal lavage[105]. Furthermore, antibiotics may reduce postoperative relapse[106], postoperative pouchitis[107] and fistula related complications[108]. There has been particular interest recently in polymorphisms in the CARD15/NOD2 gene, an intracellular bacterial pattern recognition receptor, as a risk factor for the development of Crohn's disease[109]. Probiotics may therefore exert benefits in IBD management by modulatory effects on intestinal flora. For example *Lactobacillus* GG, when administered to children with Crohn's disease, increased mucosal IgA levels[41] improved intestinal permeability and reduced disease activity[110]. Further, the relapse rate in 32 adults with inactive Crohn's disease was reduced to 6% when subjects in remission were treated with mesalazine and *S. boulardii* as compared to 38% with mesalazine alone[111]. However, in a placebo controlled study of 37 Crohn's disease patients treated with *Lactobacillus* GG for 12 mo after curative resection, the probiotic did not prevent relapse: in fact more severe endoscopic findings were reported in the *Lactobacillus* group[112].

Recent clinical studies have evaluated the effect of non-pathogenic *E. coli* strain Nissle 1917 versus mesalazine for maintenance of remission in ulcerative colitis. Kruis *et al*[113] studied 120 and Rembacken *et al*[114] 116 patients with inactive ulcerative colitis over a period of 12 wk and 1 year respectively given either *E. coli* strain Nissle 1917 (MutaflorR) or mesalazine. These unblinded studies found a similar relapse) in both groups (73% in the mesalazine group and 67% in the *E. coli* group), suggesting that probiotic therapy may be an alternative maintenance therapy. Similar results were suggested in a third RCT study[115]. 327 patients were treated with mesalazine or *E. coli* Nissle 1917 for twelve months. Relapse rates were similar (45.1% in the probiotic group versus 36.4% in the mesalazine group). In a controlled trial by Tursi *et al*[116], low dose balsalazide with VSL#3 was shown to be more effective than balsalazide or mesalazine alone in patients with acute mild to moderate ulcerative colitis. The combination of a prebiotic and a probiotic (*Bifidobacterium longum*/Synergy 1) was associated with improvement in histological scores and measures of immune activation in a randomised controlled pilot study[117].

A recent study investigated the expression and function of CARD15/NOD2 in intestinal epithelial cell lines CARD15/NOD2 mRNA was expressed in both intestinal epithelial cell lines and primary intestinal epithelial cells. CARD15/NOD2 mRNA and protein were up-regulated by tumor necrosis factor alpha (TNF alpha) in SW480 cells. This study suggests that CARD15/NOD2 may serve as a key component of innate mucosal responses to luminal bacteria as an antibacterial factor[109]. Failure in this activity may contribute to the development of Crohn's disease.

Probiotics in pouchitis

Pouchitis is an inflammation of an ileal reservoir surgically constructed in the management of IBD. It is associated with reduced counts of *Bifidobacteria* and *Lactobacilli* with increased numbers of *Clostridia* and anaerobes in faecal samples[118]. Increases in bile acids and decreases in short chain fatty acids, with a net increase in pH, may also be seen[118]. In a double blind randomized placebo controlled trial a probiotic mixture VSL#3 was studied over 9 mo in 40 patients with chronic relapsing pouchitis[119]. Relapse was defined by a pouch disease activity index (PDAI)[120] of 2 points or more and confirmed by endoscopy and histology, and was, strikingly, only identified in 15% of the VSL#3 group as against 100% in the placebo group. In a prophylactic study, 2 of 20 patients (10%) receiving 1 packet of VSL#3 1 year developed pouchitis, versus 40% of placebo treated patients[121].

Somewhat different conclusions were reached in two recently published studies[122,123]. In a group of 36 patients with recurrent or refractory pouchitis who had required antibiotics at least twice in the past year, patients were randomly assigned to VSL#3 or placebo after achieving remission, once daily for a year. More patients remained in remission in the probiotic group (85% *vs* 6%)[122].

In an observational study involving 31 patients treated with VSL#3 after achieving remission with Ciprofloxacin, only a minority of patients remained on probiotic therapy and in sustained remission, having stopped them due to recurrence of disease or adverse effects[123]. In summary then, the benefit of probiotics in Crohn's disease remains unproven. The benefit of probiotics in ulcerative colitis remains unproven. *E. coli* Nissle 1917 appears promising and may be of value in patients intolerant of or resistant to 5-ASA preparations. Limited data from small controlled studies would suggest that VSL#3 is a reasonable therapy in the primary and secondary prevention of pouchitis.

Probiotics in critical illness

Some advocates propose that probiotics have an important emerging role in managing critical illnesses originating in the gastrointestinal tract. In a recent study from Hungary patients with severe acute pancreatitis were randomised upon arrival to hospital to receive one week of treatment with a twice daily administration of a freeze dried preparation containing 10^9 live *L. plantarum* 299 with a substrate of 10 g oat fibre, or a similar preparation containing *Lactobacillus* which had been inactivated[124]. The study was stopped when the infection rate showed a significant difference in the two groups. This occurred when 45 patients had completed the study, 22 had received treatment with live and 23 with heat killed *L. plantarum* 299. Infected pancreatic necrosis occurred in 1 out of 22 subjects (4.5%) in the treatment group as against 7 of 23 (30%) in the heat killed group. The length of hospital stay was shorter in the live LAB group but it did not reach statistical significance due to small sample size[124].

Studies on cirrhotic patients have shown a decrease in the incidence of encephalopathy. A Chinese study recently reported 55 patients with minimal hepatic encephalopathy

who were randomised to receive a synbiotic preparation, fermentable fibre alone or placebo. Synbiotic treatment was associated with a reduction in serum ammonia, endotoxaemia, reversal of encephalopathy and improvement in Child-Turcotte-Pugh score in 50% patients[125]. Following liver transplantation, bacterial and fungal infections may occur in the first month despite extensive antibiotic treatment and selective digestive tract decontamination. A German study showed a reduction in post-transplant infective complications using probiotics[126]. Another recent study looked at the effects of a synbiotic preparation on gut barrier function and in critically ill patients admitted to the Intensive Care Unit (ICU). Ninety patients admitted to ICU were randomised (45 in each group) to receive either synbiotic preparations (*Lactobacillus acidophilus* La5, *Bifidobacterium lactis* Bb12, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* with oligofructose (as a prebiotic) or placebo. Patients in the symbiotic group had a lower incidence of potentially pathogenic bacteria (43% *vs* 75%, $P = 0.01$) and multiple organisms (39% *vs* 75%, $P = 0.01$) but there were no significant differences in both groups in terms of intestinal permeability, septic complications or mortality[127]. It should be pointed out that the study lasted only one week.

Probiotics and colon cancer

Colon cancer is one of the leading causes of death in the Western world. Dietary intake of red meat is probably associated with a higher risk whereas the intake of fruit, vegetables, fish and calcium are arguably associated with a lower risk. It is interesting that colon cancer risk is also lower in countries such as Netherlands and Finland where a larger quantity of yoghurt is consumed[128,129]. Could diet exert its effects *via* the microflora? If so, mechanisms involved might include altered metabolic activity of the intestinal microflora, binding and degradation of potential carcinogens, production of antitumorigenic or mutagenic compounds and enhancement in host immune response. In animals where colon cancer was induced by chemical carcinogens, administration of lactic acid bacteria resulted in a suppression of DNA damage, tumour formation and growth[130-133].

One bio-epidemiological study showed a higher risk of colon cancer in the simple presence of *Bacteroides* species, but lower risk with *Lactobacillus acidophilus*, *Lactobacillus* S06 and *Eubacterium aerofaciens* identifiable in faecal flora[134]. This raises the intriguing hypothesis that similarly shifting the resident bacterial populations may be accompanied by parallel reductions in neoplastic risk. However, biodiversity in the colon may merely represent an epiphenomenological consequence of the dietary and environmental risk factors listed above.

Probiotics and digestion

Probiotics may have a promising role in certain aspects of human digestion as illustrated by some interesting studies. Lactose digestion has been shown to improve in lactose malabsorbers who consume live yoghurt rather than milk[135,136]. The beneficial effects of yoghurt in lactose malabsorbers may result from improved digestion of lactose in the colon from stimulation of colonic bacterial activity by lactic acid bacteria[137].

Probiotics in irritable bowel syndrome

Irritable bowel syndrome (IBS) is a collection of functional gastrointestinal symptoms such as abdominal pain, defaecatory frequency and or constipation. This area is inevitably contentious, since it remains unclear how much intrinsic intestinal pathology exists in IBS. However, changes in gut sensitivity and defaecatory function are clearly present. Alterations in the composition of intestinal flora have been reported but not proven including a decrease in faecal *Lactobacilli*, *E. coli* and *Bifidobacteria*[41,110,111] and an increase in other faecal anaerobes[138-140]. Symptomatic improvement was noted in a very small crossover trial of 18 patients with IBS given L.acidophilus[141] and in an uncontrolled study using *E. faecium* PR88[142]. On the other hand, no improvement was seen in bloating, pain or urgency to defaecate after the consumption of *Lactobacillus* GG for 8 wk[143,144].In a study reported by Saggioro *et al*[145], 50 patients with IBS according to Rome II criteria were randomly assigned to a probiotic preparation (a combination of *Lactobacillus plantarum* LPO 1 and *Bifidobacterium breve* Bro or placebo for 4 wk). Pain and severity scores decreased significantly after 14 d of treatment. In a more recent study, 77 patients were randomly assigned to a malted drink containing *Lactobacillus* salivarus UCC4331 or *Bifidobacterium infantis* 35 624 or a malted drink alone[146]. Significant improvement in symptoms was noted in the *B. infantis* group. A corresponding normalisation in the ratio of IL-10/IL 12 was also noted suggesting that the probiotic may help reduce a proinflammatory state associated with IBS.

However the heterogeneity of the various studies makes it difficult to draw conclusions on the effect of probiotics in IBS, and the field is bedevilled by the fact that all therapeutic interventions in IBS produce a 30%-50% placebo response.

Probiotics and Helicobacter pylori

H pylori infection is associated with gastritis, gastro duodenal ulcers and gastric malignancies. The majority of *H Pylori* hosts become hypochlorhydric with time. Clinical studies and experimental models have shown that the secreted products of *Lactobacillus acidophilus* can suppress *H Pylori* growth *in vitro* and *in vivo* *L. johnsonii*[147] and LG21[148] are effective in suppressing the growth of *H pylori* and reducing gastric inflammation. Placebo controlled studies have demonstrated a reduction in side effects of standard triple therapy if probiotics were administered concurrently[149-151]. Daily intake of inactivated *L. acidophilus* was shown to improve the efficacy of eradication treatment[152]. Only one study[153] showed that supplementation with fermented milk, containing live special probiotic *L. casei* DN-114001, confers an enhanced therapeutic benefit on *H pylori* eradication in children with gastritis on triple therapy. The theory that probiotic therapy enhances the disappearance of *H pylori* does not gain any strength from the available literature. Further clinical studies would be needed to evaluate the effects of long term ingestion of probiotics in preventing Helicobacter-associated diseases, but are unlikely to supplant *H pylori* eradication which is rapidly and

highly effective.

In conclusion probiotics are live microbial food supplements or components of bacteria which may have beneficial effects on gastrointestinal health. Innate bacterial floras clearly play an important role in reinforcement of the physical gut barrier, affecting paracellular permeability, mucosal trophic action and microbiological interactions with mucosal lining of the gut. The key and unanswered question is whether the deliberate manipulation of the bacterial complement in the gut can confer clinical benefit. Probiotics do now appear to have a potential role in the prevention and treatment of various gastrointestinal illnesses, but it is likely that benefits achieved are specific to the bacterial species used and to the underlying disease context. Much further work is required from bench to bedside before we can realise the potential of these new interventions.

REFERENCES

1 **Metchnikoff E**. The prolongation of life. Optimistic studies New York: Putman's Sons, 1908: 161-183
2 **Lilly DM**, Stilwell RH. Growth promoting factors produced by probiotics. *Science* 1965; **147**: 747-748
3 **Parker RB**. Probiotics, the other half of the antibiotic story. *Animal nutr Health* 1974; **29**: 4-8
4 **Fuller R**. Probiotics in man and animals. *J Appl Bacteriol* 1989; **66**: 365-378
5 **Salminen S**, Bouley C, Boutron-Ruault MC, Cummings JH, Franck A, Gibson GR, Isolauri E, Moreau MC, Roberfroid M, Rowland I. Functional food science and gastrointestinal physiology and function. *Br J Nutr* 1998; **80** Suppl 1: S147-S171
6 **Marteau P**, Cuillerier E, Meance S, Gerhardt MF, Myara A, Bouvier M, Bouley C, Tondu F, Bommelaer G, Grimaud JC. Bifidobacterium animalis strain DN-173 010 shortens the colonic transit time in healthy women: a double-blind, randomized, controlled study. *Aliment Pharmacol Ther* 2002; **16**: 587-593
7 **Charteris WP**, Kelly PM, Morelli L, Collins JK. Selective detection, enumeration and identification of potentially probiotic Lactobacillus and Bifidobacterium species in mixed bacterial populations. *Int J Food Microbiol* 1997; **35**: 1-27
8 **Gibson GR**, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 1995; **125**: 1401-1412
9 **Long SS**, Swenson RM. Development of anaerobic fecal flora in healthy newborn infants. *J Pediatr* 1977; **91**: 298-301
10 **Yoshioka H**, Iseki K, Fujita K. Development and differences of intestinal flora in the neonatal period in breast-fed and bottle-fed infants. *Pediatrics* 1983; **72**: 317-321
11 **Harmsen HJ**, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, Bindels JG, Welling GW. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr* 2000; **30**: 61-67
12 **Grönlund MM**, Lehtonen OP, Eerola E, Kero P. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. *J Pediatr Gastroenterol Nutr* 1999; **28**: 19-25
13 **Simhon A**, Douglas JR, Drasar BS, Soothill JF. Effect of feeding on infants' faecal flora. *Arch Dis Child* 1982; **57**: 54-58
14 **Lundequist B**, Nord CE, Winberg J. The composition of the faecal microflora in breastfed and bottle fed infants from birth to eight weeks. *Acta Paediatr Scand* 1985; **74**: 45-51
15 **Adlerberth I**, Carlsson B, de Man P, Jalil F, Khan SR, Larsson P, Mellander L, Svanborg C, Wold AE, Hanson LA. Intestinal colonization with Enterobacteriaceae in Pakistani and Swedish hospital-delivered infants. *Acta Paediatr Scand* 1991; **80**: 602-610
16 **Hooper LV**, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host-microbial relationships in the intestine. *Science* 2001; **291**: 881-884
17 **Ducluzeau R**. [Development, equilibrium and role of microbial flora in the newborn]. *Ann Pediatr* (Paris) 1993; **40**: 13-22
18 **Ouwehand A**, Vesterlund S. Health aspects of probiotics. *IDrugs* 2003; **6**: 573-580
19 **Mitic LL**, Van Itallie CM, Anderson JM. Molecular physiology and pathophysiology of tight junctions I. Tight junction structure and function: lessons from mutant animals and proteins. *Am J Physiol Gastrointest Liver Physiol* 2000; **279**: G250-G254
20 **Stuart RO**, Nigam SK. Regulated assembly of tight junctions by protein kinase C. *Proc Natl Acad Sci USA* 1995; **92**: 6072-6076
21 **Berkes J**, Viswanathan VK, Savkovic SD, Hecht G. Intestinal epithelial responses to enteric pathogens: effects on the tight junction barrier, ion transport, and inflammation. *Gut* 2003; **52**: 439-451
22 **Bjarnason I**, MacPherson A, Hollander D. Intestinal permeability: an overview. *Gastroenterology* 1995; **108**: 1566-1581
23 **Kindon H**, Pothoulakis C, Thim L, Lynch-Devaney K, Podolsky DK. Trefoil peptide protection of intestinal epithelial barrier function: cooperative interaction with mucin glycoprotein. *Gastroenterology* 1995; **109**: 516-523
24 **García-Lafuente A,** Antolín M, Guarner F, Crespo E, Malagelada JR. Modulation of colonic barrier function by the composition of the commensal flora in the rat. *Gut* 2001; **48**: 503-507
25 **Madsen K,** Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C, Doyle J, Jewell L, De Simone C. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology* 2001; **121**: 580-591
26 **Otte JM**, Podolsky DK. Functional modulation of enterocytes by gram-positive and gram-negative microorganisms. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G613- G626
27 **Janeway CA Jr**, Medzhitov R. Innate immune recognition. *Annu Rev Immunol* 2002; **20**: 197-216
28 **Cario E,** Rosenberg IM, Brandwein SL, Beck PL, Reinecker HC, Podolsky DK. Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. *J Immunol* 2000; **164**: 966-972
29 **Cario E**, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect Immun* 2000; **68**: 7010-7017
30 **Gewirtz AT**, Navas TA, Lyons S, Godowski PJ, Madara JL. Cutting edge: bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. *J Immunol* 2001; **167**: 1882-1885
31 **Abreu MT**, Vora P, Faure E, Thomas LS, Arnold ET, Arditi M. Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide. *J Immunol* 2001; **167**: 1609-1616
32 **Fusunyan RD**, Nanthakumar NN, Baldeon ME, Walker WA. Evidence for an innate immune response in the immature human intestine: toll-like receptors on fetal enterocytes. *Pediatr Res* 2001; **49**: 589-593
33 **Haller D**, Russo MP, Sartor RB, Jobin C. IKK beta and phosphatidylinositol 3-kinase/Akt participate in non-pathogenic Gram-negative enteric bacteria-induced RelA phosphorylation and NF-kappa B activation in both primary and intestinal epithelial cell lines. *J Biol Chem* 2002; **277**: 38168-38178
34 **Cario E**, Brown D, McKee M, Lynch-Devaney K, Gerken G, Podolsky DK. Commensal-associated molecular patterns induce selective toll-like receptor-trafficking from apical membrane to cytoplasmic compartments in polarized intestinal epithelium. *Am J Pathol* 2002; **160**: 165-173
35 **Cario E**, Gerken G, Podolsky DK. Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity via protein kinase C. *Gastroenterology* 2004; **127**: 224-238
36 **Isolauri E**, Sütas Y, Kankaanpää P, Arvilommi H, Salminen S. Probiotics: effects on immunity. *Am J Clin Nutr* 2001; **73**: 444S-450S
37 **Fukushima Y**, Kawata Y, Mizumachi K, Kurisaki J, Mitsuoka T. Effect of bifidobacteria feeding on fecal flora and production of immunoglobulins in lactating mouse. *Int J Food Microbiol* 1999; **46**: 193-197

38 **Rodrigues AC**, Cara DC, Fretez SH, Cunha FQ, Vieira EC, Nicoli JR, Vieira LQ. Saccharomyces boulardii stimulates sIgA production and the phagocytic system of gnotobiotic mice. *J Appl Microbiol* 2000; **89**: 404-414

39 **Shu Q**, Gill HS. A dietary probiotic (Bifidobacterium lactis HN019) reduces the severity of Escherichia coli O157:H7 infection in mice. *Med Microbiol Immunol* 2001; **189**: 147-152

40 **Shu Q**, Gill HS. Immune protection mediated by the probiotic Lactobacillus rhamnosus HN001 (DR20) against Escherichia coli O157:H7 infection in mice. *FEMS Immunol Med Microbiol* 2002; **34**: 59-64

41 **Malin M**, Suomalainen H, Saxelin M, Isolauri E. Promotion of IgA immune response in patients with Crohn's disease by oral bacteriotherapy with Lactobacillus GG. *Ann Nutr Metab* 1996; **40**: 137-145

42 **Madsen KL**, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 1999; **116**: 1107-1114

43 **Schultz M,** Veltkamp C, Dieleman LA, Grenther WB, Wyrick PB, Tonkonogy SL, Sartor RB. Lactobacillus plantarum 299V in the treatment and prevention of spontaneous colitis in interleukin-10-deficient mice. *Inflamm Bowel Dis* 2002; **8**: 71-80

44 **Mao Y,** Nobaek S, Kasravi B, Adawi D, Stenram U, Molin G, Jeppsson B. The effects of Lactobacillus strains and oat fiber on methotrexate-induced enterocolitis in rats. *Gastroenterology* 1996; **111**: 334-344

45 **Isolauri E**. Probiotics in human disease. *Am J Clin Nutr* 2001; **73**: 1142S-1146S

46 **Alam M**, Midtvedt T, Uribe A. Differential cell kinetics in the ileum and colon of germfree rats. *Scand J Gastroenterol* 1994; **29**: 445-451

47 **Cummings JH**, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* 1987; **28**: 1221-1227

48 **Cummings JH**, Englyst HN. Fermentation in the human large intestine and the available substrates. *Am J Clin Nutr* 1987; **45**: 1243-1255

49 **Frankel WL**, Zhang W, Singh A, Klurfeld DM, Don S, Sakata T, Modlin I, Rombeau JL. Mediation of the trophic effects of short-chain fatty acids on the rat jejunum and colon. *Gastroenterology* 1994; **106**: 375-380

50 **Siavoshian S**, Segain JP, Kornprobst M, Bonnet C, Cherbut C, Galmiche JP, Blottière HM. Butyrate and trichostatin A effects on the proliferation/differentiation of human intestinal epithelial cells: induction of cyclin D3 and p21 expression. *Gut* 2000; **46**: 507-514

51 **Gibson PR**, Moeller I, Kagelari O, Folino M, Young GP. Contrasting effects of butyrate on the expression of phenotypic markers of differentiation in neoplastic and non-neoplastic colonic epithelial cells in vitro. *J Gastroenterol Hepatol* 1992; **7:** 165-172

52 **Conly JM**, Stein K, Worobetz L, Rutledge-Harding S. The contribution of vitamin K2 (menaquinones) produced by the intestinal microflora to human nutritional requirements for vitamin K. *Am J Gastroenterol* 1994; **89**: 915-923

53 **Hill MJ**. Intestinal flora and endogenous vitamin synthesis. *Eur J Cancer Prev* 1997; **6** Suppl 1: S43-S45

54 **Roberfroid MB**, Bornet F, Bouley C, Cummings JH. Colonic microflora: nutrition and health. Summary and conclusions of an International Life Sciences Institute (ILSI) [Europe] workshop held in Barcelona, Spain. *Nutr Rev* 1995; **53**: 127-130

55 **Miyazawa E**, Iwabuchi A, Yoshida T. Phytate breakdown and apparent absorption of phosphorus, calcium and magnesium in germ free and conventionalized rats. *Nutr Res* 1996; **16**: 603-613

56 **Younes H**, Coudray C, Bellanger J, Demigné C, Rayssiguier Y, Rémésy C. Effects of two fermentable carbohydrates (inulin and resistant starch) and their combination on calcium and magnesium balance in rats. *Br J Nutr* 2001; **86**: 479-485

57 **Kirjavainen PV**, Ouwehand AC, Isolauri E, Salminen SJ. The ability of probiotic bacteria to bind to human intestinal mucus. *FEMS Microbiol Lett* 1998; **167**: 185-189

58 **Jin LZ**, Marquardt RR, Zhao X. A strain of Enterococcus faecium (18C23) inhibits adhesion of enterotoxigenic Escherichia coli K88 to porcine small intestine mucus. *Appl Environ Microbiol* 2000; **66**: 4200-4204

59 **Isolauri E**, Juntunen M, Rautanen T, Sillanaukee P, Koivula T. A human Lactobacillus strain (Lactobacillus casei sp strain GG) promotes recovery from acute diarrhea in children. *Pediatrics* 1991; **88**: 90-97

60 **Majamaa H**, Isolauri E, Saxelin M, Vesikari T.Lactic acid bacteria in the treatment of acute rotavirus gastroenteritis. *J Pediatr Gastroenterol Nutr* 1995; **20**: 333-338

61 **Pant AR,** Graham SM, Allen SJ, Harikul S, Sabchareon A, Cuevas L, Hart CA. Lactobacillus GG and acute diarrhoea in young children in the tropics. *J Trop Pediatr* 1996; **42**: 162-165

62 **Isolauri E**, Kaila M, Mykkänen H, Ling WH, Salminen S. Oral bacteriotherapy for viral gastroenteritis. *Dig Dis Sci* 1994; **39**: 2595-2600

63 **Isolauri E,** Kaila M, Arvola T, Majamaa H, Rantala I, Virtanen E, Arvilommi H. Diet during rotavirus enteritis affects jejunal permeability to macromolecules in suckling rats. *Pediatr Res* 1993; **33**: 548-553

64 **Guarino A**, Canani RB, Spagnuolo MI, Albano F, Di Benedetto L. Oral bacterial therapy reduces the duration of symptoms and of viral excretion in children with mild diarrhea. *J Pediatr Gastroenterol Nutr* 1997; **25**: 516-519

65 **Guandalini S,** Pensabene L, Zikri MA, Dias JA, Casali LG, Hoekstra H, Kolacek S, Massar K, Micetic-Turk D, Papadopoulou A, de Sousa JS, Sandhu B, Szajewska H, Weizman Z. Lactobacillus GG administered in oral rehydration solution to children with acute diarrhea: a multicenter European trial. *J Pediatr Gastroenterol Nutr* 2000; **30**: 54-60

66 **Saavedra JM**, Bauman NA, Oung I, Perman JA, Yolken RH. Feeding of Bifidobacterium bifidum and Streptococcus thermophilus to infants in hospital for prevention of diarrhoea and shedding of rotavirus. *Lancet* 1994; **344**: 1046-1049

67 **Oberhelman RA,** Gilman RH, Sheen P, Taylor DN, Black RE, Cabrera L, Lescano AG, Meza R, Madico G. A placebo-controlled trial of Lactobacillus GG to prevent diarrhea in undernourished Peruvian children. *J Pediatr* 1999; **134**: 15-20

68 **Szajewska H**, Kotowska M, Mrukowicz JZ, Armańska M, Mikołajczyk W. Efficacy of Lactobacillus GG in prevention of nosocomial diarrhea in infants. *J Pediatr* 2001; **138**: 361-365

69 **Chapoy P**. [Treatment of acute infantile diarrhea: controlled trial of Saccharomyces boulardii]. *Ann Pediatr* (Paris) 1985; **32**: 561-563

70 **Hochter W**, Chase D, Hagenhoff G. Saccharomyces boulardii bei acuter Erwachsenen diarrhoea Wirkamkeit and Vertraglichkeit der Behandlung. *Munchen Medizinische Wochenschr* 1990; 188-192

71 **Buydens P**, Debeuckelaere S. Efficacy of SF 68 in the treatment of acute diarrhea. A placebo-controlled trial. *Scand J Gastroenterol* 1996; **31**: 887-891

72 **Mitra AK**, Rabbani GH. A double-blind, controlled trial of bioflorin (Streptococcus faecium SF68) in adults with acute diarrhea due to Vibrio cholerae and enterotoxigenic Escherichia coli. *Gastroenterology* 1990; **99**: 1149-1152

73 **Wunderlich PF,** Braun L, Fumagalli I, D'Apuzzo V, Heim F, Karly M, Lodi R, Politta G, Vonbank F, Zeltner L. Double-blind report on the efficacy of lactic acid-producing Enterococcus SF68 in the prevention of antibiotic-associated diarrhoea and in the treatment of acute diarrhoea. *J Int Med Res* 1989; **17**: 333-338

74 **McFarland LV**. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. *Dig Dis* 1998; **16**: 292-307

75 **McFarland LV**. Epidemiology of infectious and iatrogenic nosocomial diarrhea in a cohort of general medicine patients. *Am J Infect Control* 1995; **23**: 295-305

76 **Adam J**, Barret A, Barret-Bellet C. Essais cliniques controles en doubles inso de lultra-leur lyophyilisec. Etude multi-centrique par 25; medicins de 388 cas. *Gaz Med Francaise* 1977; **84:** 2072-2078

77 **Arvola T**, Laiho K, Torkkeli S, Mykkänen H, Salminen S, Maunula L, Isolauri E. Prophylactic Lactobacillus GG reduces antibiotic-associated diarrhea in children with respiratory in-

fections: a randomized study. *Pediatrics* 1999; **104**: e64
78 **Borgia M**, Sepe N, Brancaro V, Simone P, Borgia R. A controlled clinical study on Streptococcus faecium preparation for the prevention of side reactions during long term antibiotic treatments. *Curr Ther Res* 1982; **1**: 265-271
79 **Gotz V**, Romankiewicz JA, Moss J, Murray HW. Prophylaxis against ampicillin-associated diarrhea with a lactobacillus preparation. *Am J Hosp Pharm* 1979; **36**: 754-757
80 **Siitonen S,** Vapaatalo H, Salminen S, Gordin A, Saxelin M, Wikberg R, Kirkkola AL. Effect of Lactobacillus GG yoghurt in prevention of antibiotic associated diarrhoea. *Ann Med* 1990; **22**: 57-59
81 **McFarland LV**, Surawicz CM, Greenberg RN, Elmer GW, Moyer KA, Melcher SA, Bowen KE, Cox JL. Prevention of beta-lactam-associated diarrhea by Saccharomyces boulardii compared with placebo. *Am J Gastroenterol* 1995; **90**: 439-448
82 **Vanderhoof JA**, Whitney DB, Antonson DL, Hanner TL, Lupo JV, Young RJ. Lactobacillus GG in the prevention of antibiotic-associated diarrhea in children. *J Pediatr* 1999; **135**: 564-568
83 **Thomas MR**, Litin SC, Osmon DR, Corr AP, Weaver AL, Lohse CM. Lack of effect of Lactobacillus GG on antibiotic-associated diarrhea: a randomized, placebo-controlled trial. *Mayo Clin Proc* 2001; **76**: 883-889
84 **Lewis SJ**, Potts LF, Barry RE. The lack of therapeutic effect of Saccharomyces boulardii in the prevention of antibiotic-related diarrhoea in elderly patients. *J Infect* 1998; **36**: 171-174
85 **Tankanow RM**, Ross MB, Ertel IJ, Dickinson DG, McCormick LS, Garfinkel JF. A double-blind, placebo-controlled study of the efficacy of Lactinex in the prophylaxis of amoxicillin-induced diarrhea. *DICP* 1990; **24**: 382-384
86 **Cremonini F**, Di Caro S, Nista EC, Bartolozzi F, Capelli G, Gasbarrini G, Gasbarrini A. Meta-analysis: the effect of probiotic administration on antibiotic-associated diarrhoea. *Aliment Pharmacol Ther* 2002; **16**: 1461-1467
87 **D'Souza AL**, Rajkumar C, Cooke J, Bulpitt CJ. Probiotics in prevention of antibiotic associated diarrhoea: meta-analysis. *BMJ* 2002; **324**: 1361
88 **Fekety R**, McFarland LV, Surawicz CM, Greenberg RN, Elmer GW, Mulligan ME. Recurrent Clostridium difficile diarrhea: characteristics of and risk factors for patients enrolled in a prospective, randomized, double-blinded trial. *Clin Infect Dis* 1997; **24**: 324-333
89 **Aronsson B**, Barany P, Nord CE, Nyström B, Stenvinkel P. Clostridium difficile-associated diarrhoea in uremic patients. *Eur J Clin Microbiol* 1987; **6**: 352-356
90 **Gorbach SL**, Chang TW, Goldin B. Successful treatment of relapsing Clostridium difficile colitis with Lactobacillus GG. *Lancet* 1987; **2**: 1519
91 **Biller JA**, Katz AJ, Flores AF, Buie TM, Gorbach SL. Treatment of recurrent Clostridium difficile colitis with Lactobacillus GG. *J Pediatr Gastroenterol Nutr* 1995; **21**: 224-226
92 **Bennet RG**, Laughton B, Lindsay J et al. Lactobacillus GG treatment of Clostridium difficile infection in nursing home patients. Abstract of the 3rd International Conference on Nosocomial Infection, Atlanta, Georgia 1990
93 **Pochapin M**. The effect of probiotics on Clostridium difficile diarrhea. *Am J Gastroenterol* 2000; **95**: S11-S13
94 **Surawicz CM**, McFarland LV, Elmer G, Chinn J. Treatment of recurrent Clostridium difficile colitis with vancomycin and Saccharomyces boulardii. *Am J Gastroenterol* 1989; **84**: 1285-1287
95 **Popoola J**, Swann A, Warwick G. Clostridium difficile in patients with renal failure - management of an outbreak using biotherapy. *Nephrol Dial Transplant* 2000; **15**: 571-574
96 **McFarland LV**, Surawicz CM, Greenberg RN, Fekety R, Elmer GW, Moyer KA, Melcher SA, Bowen KE, Cox JL, Noorani Z. A randomized placebo-controlled trial of Saccharomyces boulardii in combination with standard antibiotics for Clostridium difficile disease. *JAMA* 1994; **271**: 1913-1918
97 **Surawicz CM,** McFarland LV, Greenberg RN, Rubin M, Fekety R, Mulligan ME, Garcia RJ, Brandmarker S, Bowen K, Borjal D, Elmer GW. The search for a better treatment for recurrent Clostridium difficile disease: use of high-dose vancomycin combined with Saccharomyces boulardii. *Clin Infect Dis* 2000; **31**: 1012-1017
98 **Dendukuri N,** Costa V, McGregor M, Brophy JM. Probiotic therapy for the prevention and treatment of Clostridium difficile-associated diarrhea: a systematic review. *CMAJ* 2005; **173**: 167-170
99 **Muñoz P,** Bouza E, Cuenca-Estrella M, Eiros JM, Pérez MJ, Sánchez-Somolinos M, Rincón C, Hortal J, Peláez T. Saccharomyces cerevisiae fungemia: an emerging infectious disease. *Clin Infect Dis* 2005; **40**: 1625-1634
100 **Podolsky DK**. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429
101 **Rath HC,** Herfarth HH, Ikeda JS, Grenther WB, Hamm TE Jr, Balish E, Taurog JD, Hammer RE, Wilson KH, Sartor RB. Normal luminal bacteria, especially Bacteroides species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/human beta2 microglobulin transgenic rats. *J Clin Invest* 1996; **98**: 945-953
102 **Rath HC**, Schultz M, Dieleman LA, Li F, Kolbl H, Falk W, Scholmerich J, Sartor RB. Selective vs. broad spectrum antibiotics in the prevention and treatment of experimental colitis in two rodent models. *Gastroenterology* 1998; **114**: A1067
103 **Rutgeerts P**, Goboes K, Peeters M, Hiele M, Penninckx F, Aerts R, Kerremans R, Vantrappen G.Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. *Lancet* 1991; **338**: 771-774
104 **Janowitz HD**, Croen EC, Sachar DB. The role of the fecal stream in Crohn's disease: an historical and analytic review. *Inflamm Bowel Dis* 1998; **4**: 29-39
105 **Wellmann W**, Fink PC, Benner F, Schmidt FW. Endotoxaemia in active Crohn's disease. Treatment with whole gut irrigation and 5-aminosalicylic acid. *Gut* 1986; **27**: 814-820
106 **Rutgeerts P**, Hiele M, Geboes K, Peeters M, Penninckx F, Aerts R, Kerremans R. Controlled trial of metronidazole treatment for prevention of Crohn's recurrence after ileal resection. *Gastroenterology* 1995; **108**: 1617-1621
107 **Madden MV**, McIntyre AS, Nicholls RJ. Double-blind crossover trial of metronidazole versus placebo in chronic unremitting pouchitis. *Dig Dis Sci* 1994; **39**: 1193-1196
108 **Solomon MJ**, McLeod RS, O'Connor BI et al. Combination ciprofloxacin and metronidazole in severe perianal Crohn's disease. *Can J Gastroenterol* 1993; **7**: 571-573
109 **Hisamatsu T**, Suzuki M, Reinecker HC, Nadeau WJ, McCormick BA, Podolsky DK. CARD15/NOD2 functions as an antibacterial factor in human intestinal epithelial cells. *Gastroenterology* 2003; **124**: 993-1000
110 **Gupta P**, Andrew H, Kirschner BS, Guandalini S. Is lactobacillus GG helpful in children with Crohn's disease? Results of a preliminary, open-label study. *J Pediatr Gastroenterol Nutr* 2000; **31**: 453-457
111 **Guslandi M**, Mezzi G, Sorghi M, Testoni PA. Saccharomyces boulardii in maintenance treatment of Crohn's disease. *Dig Dis Sci* 2000; **45**: 1462-1464
112 **Prantera C**, Scribano ML, Falasco G, Andreoli A, Luzi C. Ineffectiveness of probiotics in preventing recurrence after curative resection for Crohn's disease: a randomised controlled trial with Lactobacillus GG. *Gut* 2002; **51**: 405-409
113 **Kruis W**, Schütz E, Fric P, Fixa B, Judmaier G, Stolte M. Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 1997; **11**: 853-858
114 **Rembacken BJ**, Snelling AM, Hawkey PM, Chalmers DM, Axon AT. Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial. *Lancet* 1999; **354**: 635-639
115 **Kruis W,** Fric P, Pokrotnieks J, Lukás M, Fixa B, Kascák M, Kamm MA, Weismueller J, Beglinger C, Stolte M, Wolff C, Schulze J. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. *Gut* 2004; **53**: 1617-1623
116 **Tursi A**, Brandimarte G, Giorgetti GM, Forti G, Modeo ME, Gigliobianco A. Low-dose balsalazide plus a high-potency probiotic preparation is more effective than balsalazide alone

or mesalazine in the treatment of acute mild-to-moderate ulcerative colitis. *Med Sci Monit* 2004; **10**: PI126-PI131
117 **Furrie E**, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'neil DA, Macfarlane GT. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 2005; **54**: 242-249
118 **Ruseler-van Embden JG**, Schouten WR, van Lieshout LM. Pouchitis: result of microbial imbalance? *Gut* 1994; **35**: 658-664
119 **Gionchetti P,** Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, Poggioli G, Miglioli M, Campieri M. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology* 2000; **119**: 305-309
120 **Sandborn WJ,** Tremaine WJ, Batts KP, Pemberton JH, Phillips SF. Pouchitis after ileal pouch-anal anastomosis: a Pouchitis Disease Activity Index. *Mayo Clin Proc* 1994; **69**: 409-415
121 **Gionchetti P,** Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, Vitali B, Poggioli G, Miglioli M, Campieri M. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. *Gastroenterology* 2003; **124**: 1202-1209
122 **Mimura T,** Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot IC, Nicholls RJ, Gionchetti P, Campieri M, Kamm MA. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut* 2004; **53**: 108-114
123 **Shen B**, Brzezinski A, Fazio VW, Remzi FH, Achkar JP, Bennett AE, Sherman K, Lashner BA. Maintenance therapy with a probiotic in antibiotic-dependent pouchitis: experience in clinical practice. *Aliment Pharmacol Ther* 2005; **22**: 721-728
124 **Oláh A,** Belágyi T, Issekutz A, Gamal ME, Bengmark S. Randomized clinical trial of specific lactobacillus and fibre supplement to early enteral nutrition in patients with acute pancreatitis. *Br J Surg* 2002; **89**: 1103-1107
125 **Liu Q**, Duan ZP, Ha DK, Bengmark S, Kurtovic J, Riordan SM. Synbiotic modulation of gut flora: effect on minimal hepatic encephalopathy in patients with cirrhosis. *Hepatology* 2004; **39**: 1441-1449
126 **Rayes N**, Seehofer D, Hansen S, Boucsein K, Müller AR, Serke S, Bengmark S, Neuhaus P. Early enteral supply of lactobacillus and fiber versus selective bowel decontamination: a controlled trial in liver transplant recipients. *Transplantation* 2002; **74**: 123-127
127 **Jain PK**, McNaught CE, Anderson AD, MacFie J, Mitchell CJ. Influence of synbiotic containing Lactobacillus acidophilus La5, Bifidobacterium lactis Bb 12, Streptococcus thermophilus, Lactobacillus bulgaricus and oligofructose on gut barrier function and sepsis in critically ill patients: a randomised controlled trial. *Clin Nutr* 2004; **23**: 467-475
128 **Shahani KM**, Ayebo AD. Role of dietary lactobacilli in gastrointestinal microecology. *Am J Clin Nutr* 1980; **33**: 2448-2457
129 **Malhotra SL**. Dietary factors in a study of cancer colon from Cancer Registry, with special reference to the role of saliva, milk and fermented milk products and vegetable fibre. *Med Hypotheses* 1977; **3**: 122-126
130 **Pool-Zobel BL,** Neudecker C, Domizlaff I, Ji S, Schillinger U, Rumney C, Moretti M, Vilarini I, Scassellati-Sforzolini R, Rowland I. Lactobacillus- and bifidobacterium-mediated antigenotoxicity in the colon of rats. *Nutr Cancer* 1996; **26**: 365-380
131 **Reddy BS**, Rivenson A. Inhibitory effect of Bifidobacterium longum on colon, mammary, and liver carcinogenesis induced by 2-amino-3-methylimidazo[4,5-f]quinoline, a food mutagen. *Cancer Res* 1993; **53**: 3914-3918
132 **Sekine K**, Toida T, Saito M, Kuboyama M, Kawashima T, Hashimoto Y. A new morphologically characterized cell wall preparation (whole peptidoglycan) from Bifidobacterium infantis with a higher efficacy on the regression of an established tumor in mice. *Cancer Res* 1985; **45**: 1300-1307
133 **Reddy BS**. Prevention of colon cancer by pre- and probiotics: evidence from laboratory studies. *Br J Nutr* 1998; **80**: S219-S223
134 **Moore WE**, Moore LH. Intestinal floras of populations that have a high risk of colon cancer. *Appl Environ Microbiol* 1995; **61**: 3202-3207
135 **de Vrese M**, Stegelmann A, Richter B, Fenselau S, Laue C, Schrezenmeir J. Probiotics--compensation for lactase insufficiency. *Am J Clin Nutr* 2001; **73**: 421S-429S
136 **Gaudichon C**, Mahe S, Roos N, Benamouzig R, Luengo C, Huneau JF, Sick H, Bouley C, Rautureau J, Tome D. Exogenous and endogenous nitrogen flow rates and level of protein hydrolysis in the human jejunum after [15N]milk and [15N]yoghurt ingestion. *Br J Nutr* 1995; **74**: 251-260
137 **Marteau P,** Pochart P, Flourié B, Pellier P, Santos L, Desjeux JF, Rambaud JC. Effect of chronic ingestion of a fermented dairy product containing Lactobacillus acidophilus and Bifidobacterium bifidum on metabolic activities of the colonic flora in humans. *Am J Clin Nutr* 1990; **52**: 685-688
138 **Balsari A**, Ceccarelli A, Dubini F, Fesce E, Poli G. The fecal microbial population in the irritable bowel syndrome. *Microbiologica* 1982; **5**: 185-194
139 **Wyatt GM**, Bayliss CE, Lakey AF, Bradley HK, Hunter JO, Jones VA. The faecal flora of two patients with food-related irritable bowel syndrome during challenge with symptom-provoking foods. *J Med Microbiol* 1988; **26**: 295-299
140 **Bradley HK**, Wyatt GM, Bayliss CE, Hunter JO. Instability in the faecal flora of a patient suffering from food-related irritable bowel syndrome. *J Med Microbiol* 1987; **23**: 29-32
141 **Halpern GM**, Prindiville T, Blankenburg M, Hsia T, Gershwin ME. Treatment of irritable bowel syndrome with Lacteol Fort: a randomized, double-blind, cross-over trial. *Am J Gastroenterol* 1996; **91**: 1579-1585
142 **Hunter JO**, Lee AJ, King TS, Barratt MEJ, Linggood MA, Blades JA. Enterococcus faecium strain PR88: An effective probiotic. *Gut* 1996; **38**: A 62
143 **Sen S,** Mullan MM, Parker TJ, Woolner JT, Tarry SA, Hunter JO. Effect of Lactobacillus plantarum 299v on colonic fermentation and symptoms of irritable bowel syndrome. *Dig Dis Sci* 2002; **47**: 2615-2620
144 **O'Sullivan MA**, O'Morain CA. Bacterial supplementation in the irritable bowel syndrome. A randomised double-blind placebo-controlled crossover study. *Dig Liver Dis* 2000; **32**: 294-301
145 **Saggioro A**. Probiotics in the treatment of irritable bowel syndrome. *J Clin Gastroenterol* 2004; **38**: S104-S106
146 **O'Mahony L**, McCarthy J, Kelly P, Hurley G, Luo F, Chen K, O'Sullivan GC, Kiely B, Collins JK, Shanahan F, Quigley EM. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. *Gastroenterology* 2005; **128**: 541-551
147 **Felley CP**, Corthesy-Theulaz I, Rivero JL, Sipponen P, Kaufmann M, Bauerfeind P, Wiesel PH, Brassart D, Pfeifer A, Blum AL, Michetti P. Favourable effect of an acidified milk (LC-1) on Helicobacter pylori gastritis in man. *Eur J Gastroenterol Hepatol* 2001; **13**: 25-29
148 **Sakamoto I,** Igarashi M, Kimura K, Takagi A, Miwa T, Koga Y. Suppressive effect of Lactobacillus gasseri OLL 2716 (LG21) on Helicobacter pylori infection in humans. *J Antimicrob Chemother*. 2001; **47:** 709-710
149 **Cremonini F**, Di Caro S, Covino M, Armuzzi A, Gabrielli M, Santarelli L, Nista EC, Cammarota G, Gasbarrini G, Gasbarrini A. Effect of different probiotic preparations on anti-helicobacter pylori therapy-related side effects: a parallel group, triple blind, placebo-controlled study. *Am J Gastroenterol* 2002; **97**: 2744-2749
150 **Armuzzi A**, Cremonini F, Bartolozzi F, Canducci F, Candelli M, Ojetti V, Cammarota G, Anti M, De Lorenzo A, Pola P, Gasbarrini G, Gasbarrini A. The effect of oral administration of Lactobacillus GG on antibiotic-associated gastrointestinal side-effects during Helicobacter pylori eradication therapy. *Aliment Pharmacol Ther* 2001; **15**: 163-169
151 **Shimbo I**, Yamaguchi T, Odaka T, Nakajima K, Koide A, Koyama H, Saisho H. Effect of Clostridium butyricum on fecal flora in Helicobacter pylori eradication therapy. *World J Gastroenterol* 2005; **11**: 7520-7524
152 **Canducci F**, Armuzzi A, Cremonini F, Cammarota G, Bartolozzi F, Pola P, Gasbarrini G, Gasbarrini A. A lyophilized and inactivated culture of Lactobacillus acidophilus increases Heli-

cobacter pylori eradication rates. *Aliment Pharmacol Ther* 2000; **14**: 1625-1629

153 **Sykora J**, Valeckova K, Amlerova J, Siala K, Dedek P, Watkins S, Varvarovska J, Stozicky F, Pazdiora P, Schwarz J. Effects of a specially designed fermented milk product containing probiotic Lactobacillus casei DN-114 001 and the eradication of H. pylori in children: a prospective randomized double-blind study. *J Clin Gastroenterol* 2005; **39**: 692-698

S- Editor Pan BR **L- Editor** Misra SP **E- Editor** Bai SH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5458-5464
World Journal of Gastroenterology ISSN 1007-9327

REVIEW

H pylori and gastric cancer: Shifting the global burden

Christian Prinz, Susanne Schwendy, Petra Voland

Christian Prinz, Susanne Schwendy, Petra Voland, II. Medical Department, Technical University of Munich, D-81675 Munich, Germany
Supported by Deutsche Forschungsgemeinschaft (DFG Graduiertenkolleg 333: Biology of Human Diseases) and by a grant from the DFG to C.P. (Pr 411/9-1) and the Dr. Else Kröner Fresenius Stiftung
Correspondence to: Dr. Christian Prinz, Professo, Klinikum rechts der Isar, Technischen Universität München, II. Medizinische Klinik und Poliklinik, Ismaninger Str. 22, D-81675 München, Germany. christian.prinz@lrz.tum.de
Telephone: +49-89-41404073 Fax: +49-89-41407366
Received: 2006-01-04 Accepted: 2006-01-24

Abstract

Infection with *H pylori* leads to a persistent chronic inflammation of the gastric mucosa, thereby increasing the risk of distal gastric adenocarcinoma. Numerous studies have determined a clear correlation between *H pylori* infection and the risk of gastric cancer; however, general eradication is not recommended as cancer prophylaxis and time points for treatment remain controversial in different areas of the world. Prevalence rates in Western countries are decreasing, especially in younger people (< 10%); and a decline in distal gastric adenocarcinoma has been observed. Risk groups in Western countries still show considerably higher risk of developing cancer, especially in patients infected with *cagA*$^+$ strains and in persons harboring genetic polymorphism of the *IL-1B* promoter (-511T/T) and the corresponding *IL-1* receptor antagonist (*IL-1RN**2). Thus, general eradication of all infected persons in Western countries not recommended and is limited to risk groups in order to achieve a risk reduction. In contrast, infection rates and cancer prevalence are still high in East Asian countries. A prevention strategy to treat infected persons may avoid the development of gastric cancer to a large extent and with enormous clinical importance. However, studies in China and Japan indicate that prevention of gastric cancer is effective only in those patients that do not display severe histological changes such as atrophy and intestinal metaplasia. Thus, prophylactic strategies to prevent gastric cancer in high risk populations such as China should therefore especially aim at individuals now at younger age when the histological alterations caused by the bacterial infection was still reversible. In countries with a low prevalence of gastric cancer, risk groups carrying *cagA*$^+$ strains and IL-1 genetic polymorphisms should be identified and treated.

Key words: *H pylori*; Gastric cancer

Prinz C, Schwendy S, Voland P. *H pylori* and gastric cancer: Shifting the global burden. *World J Gastroenterol* 2006; 12(34): 5458-5464

http://www.wjgnet.com/1007-9327/12/5458.asp

INTRODUCTION

Since the discovery of *H pylori* in 1983, intensive research has led to the conclusion that infection with this bacterium is the major cause for the development of distal gastric cancer. Infection with the bacterium leads to a chronic inflammation of the gastric epithelium, associated with multifocal gastric atrophy, dysplasia, and malignant transformation in some of the infected patients[1-6]. Controversy remains why only a minority of infected patients develops distal adenocarcinoma, and how geographic differences between Western and Asian countries may contribute to these differences. *H pylori* infection rates average at about 30% in Western populations; in this part of the world, approximately 0.1%-1% of the patients with *H pylori* induced gastritis will develop distal gastric cancer[7,8]. Infection rates in Asian countries are higher and range at 60%-88%; distal gastric adenocarcinoma is even more frequent in these countries. Overall, gastric cancer was responsible for almost 650 000 deaths worldwide in the year 2000[9]. Death from gastric cancer is second only to lung cancer in men and thus contributes to approximately 10% of all cancer deaths annually (see: www.who.org). Since the diagnosis of early gastric cancer is difficult and in most cases diagnosis is made at a more progressed stage, treatment becomes cost-intensive. Therefore, there is a considerable interest to understand the underlining mechanisms and to find strategies to eradicate *H pylori* infection which could prevent gastric carcinogenesis. Such statistical calculations have already been anticipated 10 years ago in high risk populations of Japanese immigrants in Hawaii; currently, however, no guidelines exist when and whom to treat[10,11].

EPIDEMIOLOGICAL FEATURES IN EAST AND WEST

H pylori infection and gastric cancer in Western countries

In Germany in the year 2000, 21 000 patients suffered from gastric cancer with an equal distribution among males and females[12]. In the US (http://seer.cancer.gov),

a similar occurrence has been reported with more than 45 000 deaths per year. In countries such as the USA or Germany, the prevalence of *H pylori* is decreasing, so that meanwhile less than 10% of the children are infected with this bacterium, underlining that a prophylactic eradication or vaccination in childhood is of little interest in these countries[8,13-15]. Nevertheless, infection rates are high in elderly populations above the age of 50, and this group is particularly suffering from gastric cancer. A reduction of cancer risk may be achieved when patients are cured from the infection. It may thus be important to identify those patients who are at increased risk.

Gastric cancer in Japan and East Asia

Gastric cancer is the second highest cause of cancer-related deaths in Japan, and the death rate due to gastric adenocarcinoma has actually marginally increased to approximately 50 000 deaths per year in this country[13,14,16]. In China, a similar prevalence of gastric cancer has been reported. *H pylori* infection rates in these countries are high and range from 60% in the younger population (10-40 years old) to 80%-90% in elderly patients above the age of 50. In Japan, mortality from distal gastric adenocarcinoma is among the highest scores of the world and could be attributed to the very high rates of *H pylori* infection in this country and the fact that the most common strain of *H pylori* found in Japan is extremely virulent. A prophylactic eradication in people at younger age could save millions of lives as well as enormous costs resulting from ulcer disease, gastrointestinal bleeding and gastric cancer treatment. Therefore, a successful eradication of this pathogen is of crucial socio-economical importance especially in countries such as Japan or China, particularly especially in persons who are at age < 50 years.

The "Asian paradox"

Although there is a considerable high rate of *H pylori* infection in Asian countries such as China, Japan, Thailand and Indonesia, there is a remarkable difference regarding the outcome of gastric cancer within these countries. This observation has also been termed as the "Asian paradox": Although there is a high infection rate in Thailand and Indonesia, there is only little risk of developing gastric cancer in these countries[14,17]. As discussed below, bacterial virulence factors as well as the individual host immune response may be different among those countries and account for the differential development of the infection outcome. Questions remain whether to treat all infected patients, subgroups of patients, or wait until serious histological alterations have occurred in the various countries mentioned above. The outcome of infection, however, is difficult to predict. Recent studies have elucidated some of the mechanisms of chronic inflammation and thus enable a more precise prediction of the clinical course of infection in different countries.

Impact of strain types on development of distal gastric adenocarcinoma

In recent studies, a new analytical investigation to overcome a potential underestimation of the association of *H pylori* with gastric cancer was performed in a Western population. Applying various more stringent exclusion criteria to minimize a potential bias from this source increased the odds ratio (95% confidence interval) of non-cardia gastric cancer from 3.7 to 18.3 for any *H pylori* infection, and from 5.7 to 28.4 for *cagA*$^+$ *H pylori* infections in Germany[18-20]. A similar approach has been performed in a study from Sweden, and the results were remarkably consistent with the German study. The observations made in these studies are further supported by a recent cohort study from Japan, in which 36 out of 1246 *H pylori* infected subjects, but none of 280 uninfected subjects developed gastric cancer during a mean follow-up of 7.8 years[21]. These very stringent epidemiological studies strongly support that the *H pylori* gastric cancer association may have been strongly underestimated the risks in previous studies, and consequently, underline that infection especially with certain *cagA*$^+$ *H pylori* can be considered as a true carcinogenic factor. Determination of *cagA* status may thus help the physician to identify people who are at increased risk for gastric cancer. Meanwhile, tests have become available as a serological test and a specific antibody staining for histological specimens[16].

IMPORTANCE OF BACTERIAL STRAIN TYPES: MOLECULAR AND IMMUNOLOGICAL MECHANISMS

Bacterial virulence factors, especially genetic diversity in the *cagA* region, have been claimed to account for this diverging development. The *H pylori cag* pathogenicity island (*cag*PAI) is a 35 to 40 kb genetic element that encodes a type Ⅳ secretion system. One of the key factors of this system is the CagA protein; *cagA*$^+$ strains inject the CagA protein directly into host cells where it undergoes tyrosine phosphorylation by a host-cell kinase, and the phosphoprotein alters the physiology of the affected cells. Huang *et al* conducted a meta-analysis of the relationship between CagA seropositivity and gastric cancer[22]. Based on 7 studies with 1707 gastric cancer patients and 2124 matched controls the analysis showed that infection with *cagA*$^+$ *H pylori* strains increases the risk for gastric cancer 2.87 fold over the risk associated with *H pylori* infection alone.

Hatatekayama *et al* recently reported that CagA proteins isolated in East Asia, where gastric cancer is prevalent, have a distinct sequence at the phosphorylation site compared with CagA proteins from Western *H pylori* strains[23-25]. This CagA diversity may be one important variable in determining the biological activity of CagA and the clinical outcome of infection. Translocated CagA forms a physical complex with the SRC homology 2 domain (SH2)-containing tyrosine phosphatase (SHP-2) in a phosphorylation-dependent manner, and stimulates phosphatase activity. SHP-2 is known to play an important inductive role in mitogenic signal transduction. Deregulation of SHP-2 by CagA may induce abnormal proliferation of gastric epithelial cells. In addition, the CagA protein is polymorphic. Thus, CagA proteins isolated in northern parts of East Asia, where gastric cancer is prevalent, have a distinct sequence at the phosphorylation site and thus contribute to the differential outcome.

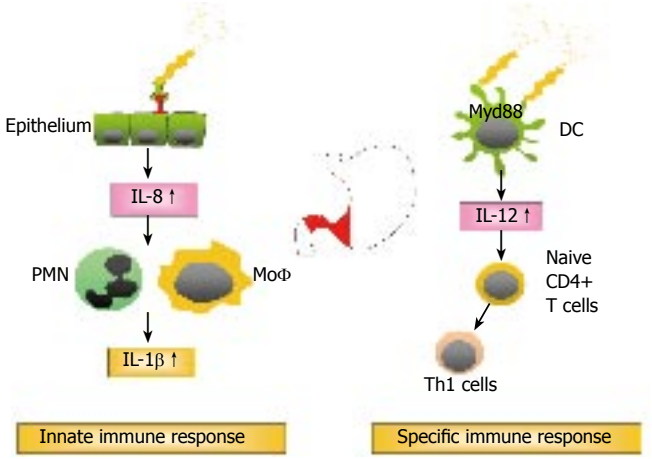


Figure 1 *H pylori*: Immune response and suppression.

Importance of bacterial adherence factors

cagA$^+$ *H pylori* strains also are more likely to express the *babA* product, which mediates adherence to Lewis^b antigens on gastric epithelial cells[26]. European studies have determined that bacteria expressing the adherence factor BabA are detected more frequently in patients with gastric cancer or severe histological changes in the mucosa. Comparing several independent studies in different countries, the relative risk for the development of distal gastric adenocarcinoma was increased up to 20-fold. These results support the hypothesis, that the risk of developing severe gastric pathologies is dramatically increased once the correct determination of the *cagA* status is performed[27-31].

Current model for the initiation of heightened gastric inflammation

A new concept has now emerged why *cagA*$^+$/*babA*$^+$ *H pylori* strains induce higher levels of the proinflammatory cytokines IL-1β and IL-8. Adherence of *H pylori* to epithelial cells *via* BabA favors a more tight adhesion to the epithelial cell and thus promotes bacterial colonisation. Injection of CagA into epithelial cells is associated with IL-8 secretion, which in turn, acts as a local chemoattractant for polymorphic mononuclear cells (PMN)[29]. These PMNs are considered to be of critical importance for the breakdown of the local epithelial barrier and may lead to a further infiltration of the bacteria into the submucosa. Thus, several studies outline the view that bacterial virulence and adherence factors contribute to the development of severe diseases in the stomach. Infection with certain *H pylori* strain types is therefore more dangerous than smoking a pack of cigarettes per day, leading to a 21-fold increased relative risk for the development of lung cancer.

Figure 1: Events during the initial infection process with H pylori

Infection with so called type 1 strains harboring *cagA*/*vacAs1* and *babA* is associated with a dramatically increased risk to develop distal gastric adenocarcinoma. Infection with specific strain types thus is the major determinant of the further sequence of events.

IMPORTANCE OF HOST GENETICS FOR THE *H PYLORI* INDUCED INFLAMMATION AND CANCER DEVELOPMENT

Genetic polymorphisms contribute to severe gastric inflammation

In addition to bacterial factors, but less important in terms of relative risk increment, are host factors that seem to influence the inflammatory response and the development of a more severe pathology. *H pylori* induced inflammation is implicated in the development of mucosal damage and is characterised by strong granulocytic and lymphocytic infiltration. The T helper cell response towards *H pylori* is generally considered to be of the Th1 phenotype, leading to a cell mediated immune response[32-35] (Figure 1). There is increasing evidence that the *H pylori* induced Th1 response contributes to cancer development. Downregulation of the Th1 response in mice by concurrent enteric helminth infection or p53 mutation was shown to protect against the development of atrophy, intestinal metaplasia, and invasive gastric carcinoma[35-37]. Important cytokines characterising Th1 mediated immune responses are interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α), and interleukin-1β (IL-1β), all being upregulated during chronic *H pylori* infection[38-40]. IL-10, which is also highly expressed in the *H pylori* infected stomach, is one of the most important regulatory cytokines, inhibiting cell mediated immune responses[38,41,42].

Genes encoding cytokines and related molecules harbour polymorphic regions, which are considered to alter gene transcription and thereby influence inflammatory processes in response to infectious diseases. Polymorphisms in the human *IL-10*, *IL-1B*, *TNF*-α, *IFN*-γ, and *IL-1* receptor antagonist (*IL-1RN*) genes have been reported to influence cytokine expression[43-47]. In the promoter region of the *IL-1B* gene, *IL-1B–511T*, which is in complete linkage disequilibrium with *IL-1B–31C*, was previously associated with slightly, but not significantly, increased IL-1β secretion from stimulated PBMC. The *IL-1RN* gene has a penta-allelic 86 bp variable number of tandem repeat region (VNTR) in intron 2, of which allele 2 (*IL-1RN*2*) was previously associated with enhanced IL-1β secretion[46] and the development of gastric cancer[44]. Presence of certain genetic polymorphisms of the host, in contrast to the bacterial factors, increase the relative risk for distal gastric adenocarcinoma by 1.5-4 fold. In a German population[48], the homozygous genotype IL-1RN*2/2 of the IL-RN gene was strongly associated with early-stage gastric cancer ($P < 0.0001$), whereas further associations with the IL-1 gene cluster were not observed. A Korean group[49] determined that patients with intestinal-type gastric cancer showed a higher frequency of IL-1B-31T homozygotes [odds ratio (OR) = 2.2; 95% confidence interval (CI) = 1.1-4.3] compared with controls. Risk was also significantly increased in these patients for IL-1B-31T homozygotes compared with patients with diffuse-type gastric cancer (OR = 3.4; 95% CI = 1.5-7.7). In a Chinese study, it was reported that the relative risks associated with the IL-1B variant genotypes were 1.64

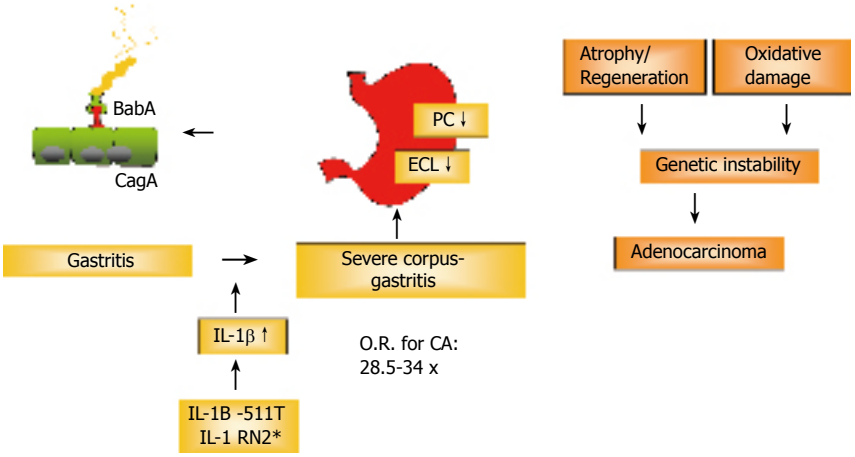


Figure 2 *H pylori*: Chronic inflammation and carcinogenesis.

(95% CI, 1.01-2.66) for -31TT and 1.52 (95% CI, 0.91-2.54) for -511CC, respectively, compared with their wild-type homozygotes[50]. The risks were significantly more evident in individuals with *H pylori* infection (adjusted OR = 2.14; 95% CI, 1.13-4.06 for -31TT; adjusted OR = 2.00; 95% CI, 1.02-3.89 for -511CC), which was consistent with the biological effects of IL-1β.

Chen *et al*[51] identified that the carriage of IL-1RN*2 polymorphism, male gender, old age and *H pylori* infection independently increased the risk of gastric cancer, with odds ratios of 3.3 (95% CI, 1.4-7.7), 2.1 (95% CI, 1.2-3.8), 5.3 (95% CI, 3.1-9.0) and 2.2 (95% CI, 1.3-3.8), respectively. *H pylori*-infected individuals who were carriers of IL-1RN*2 showed increased risks for both intestinal and diffuse types of gastric cancer, with odds ratios of 11.0 and 8.7, respectively. Thus, IL-1RN*2 was interpreted to be an independent factor governing the development of gastric cancer in Asian individuals.

In areas of high and low prevalence of gastric cancer in China[52], the IL-1B -511T/T genotype frequency was significantly higher among patients with gastric cancer (25.0%) than control subjects (12.5%) in a region with low GC prevalence. While *H pylori* infection alone had only a modest effect on the risk of gastric cancer development (OR = 5.0, 95% CI 1.5-16.3), combined with the IL-1B -511T/T genotype the risk was markedly elevated (OR = 17.1, 95% CI 3.8-76.4). The study underlines the synergy between *H pylori* infection and the host immune response to induce gastric cancer.

A recent German study confirmed this synergistic effect also in regard to the induction of premalignant changes. In a large group of *H pylori* infected patients[29], carriers of the proinflammatory IL-1B -511T/-31C and IL-1RN*2 alleles had an increased risk for the development of atrophic gastritis (AG), intestinal metaplasia (IM), and severe inflammation, ORs of 1.7 (95% CI, 0.8-3.4) to 4.4 (95% CI, 1.5-12.9). The highest prevalence of severe gastric abnormalities was found in patients with both host and bacterial high-risk genotypes (*cagA*$^+$/*vacAs1*$^+$/IL-1B -511T/IL-1RN*2), with ORs of 24.8 (95% CI, 5.2-117.3) for severe lymphocytic infiltration, 9.5 (95% CI, 2.8-32.1) for severe granulocytic infiltration, 6.0 (95% CI, 2.4-15.5) for IM, and 2.4 (95% CI, 0.93-6.2) for AG. These dramatically elevated values underline the importance of the infection for gastric cancer development. It may thus be concluded from the above studies, that combined bacterial/host genotyping thus may provide a clinical tool to identify patients at high risk of developing gastric cancer. This current concept of gastric carcinogenesis is illustrated in Figure 2.

Figure 2: Concept of gastric carcinogenesis

There is overwhelming evidence that *H pylori* infection, especially with *cagA*+ strains, leads to a strong granulocytic and lymphocytic infiltration; a subgroup of infected patients will develop gastric cancer, especially in high-risk countries such as Japan, China and East-European countries. Reasons for this progression include genetic polymorphisms, e.g. IL-1B/IL-1RN polymorphisms associated with high levels of IL-1β. IL-1β is a strong antisecretory cytokine. These genetic polymorphisms lead to a heightened cytokine release, which in turn decreases acid secretion. Subsequently, the association of hypochlorhydric conditions with the persistent inflammation in the gastric corpus is considered to be a true risk factor (RR: 34.5-fold!) for the development of gastric cancer. Studies should therefore aim not only at preventing gastric cancer, but also at preventing the development of severe corpus gastritis, leading to potentially irreversible atrophy and hypochlorhydria.

PROPHYLAXIS OF GASTRIC CANCER

Prospective intervention studies

Data from a Chinese trial[53] investigating *H pylori* eradication in gastric cancer showed that the incidence of gastric adenocarcinoma development was similar in patients undergoing eradication therapy compared with placebo over a 7.5-year period. Interestingly, in a subgroup of *H pylori*-positive patients without atrophy, intestinal metaplasia or dysplasia (pre-cancerous lesions), eradication therapy significantly decreased the development of gastric adenocarcinoma to a frequency of zero. The data are in accordance with a recent study in Japan[4]: in this prospective study, no carcinomas were observed in the *H pylori* negative group. This study supports the view that a prospective eradication study should be performed in young adults in which histological alterations have not

proceeded too far and may be reversible.

Zhou *et al*[54] investigated histopathological changes in *H pylori* eradicated subjects in China over a period of more than 5 years. The authors report in a group of 552 *H pylori*-positive subjects that, the severity and activity of inflammation in both the antrum and body were markedly reduced after *H pylori* eradication. Within the five years after eradication of *H pylori*, intestinal metaplasia in the antrum regressed or showed no progression, while the proportion of intestinal metaplasia in the *H pylori*-positive group increased significantly. After *H pylori* eradication, the atrophy in both the antrum and body had no significant regression. After eight years of observation, the authors report a significantly higher incidence of gastric cancer in the *H pylori* infected group (5/1530) than in the *H pylori*-negative (1/1230) group in the Yantai area of China, underlining the need for eradication in early stages.

Serum pepsinogen levels as risk factors for developing gastric cancer

A recently published study[55] reports that subjects seropositive for either *H pylori* or CagA who had low pepsinogen (PG) I levels had the highest OR (9.21 95% CI 4.95-17.13) for noncardia cancer, compared with subjects with neither factor. The results suggest that individuals with both *H pylori* or CagA seropositivity and a low PG Ⅰ level or low PG Ⅰ / Ⅱ ratio are highly susceptible to development of noncardia gastric cancer. Such tests can easily be performed in clinical routine. Watabe *et al*[56] have confirmed that *H pylori* infection and gastric atrophy are both risk factors for gastric cancer in a study group with a total of 9293 participants. The annual incidence of gastric cancer was 0.04%-0.60%. As expected, the highest relative risk for gastric cancer was found in *H pylori* infected subjects with gastric atrophy and high serum pepsinogen levels [OR = 8.2 (3.2-21.5)].

H pylori eradication and the risk of esophageal cancer

Concerns exist whether eradication of *H pylori* might lead to the development of esophageal cancer. To clarify the role of *H pylori* infection in these tumors with divergent incidence trends, Chow *et al*[57] analyzed serum IgG antibodies to *H pylori* and to a recombinant fragment of CagA by antigen-specific ELISA among 129 patients newly diagnosed with esophageal/gastric cardia adenocarcinoma, 67 patients with noncardia gastric adenocarcinoma, and 224 population controls. Infection with *cagA*$^+$ strains was not significantly related to risk for noncardia gastric cancers (OR = 1.4; CI = 0.7-2.8) but was significantly associated with a reduced risk for esophageal/cardia cancers (OR = 0.4; CI = 0.2-0.8). The study thus contradicts previous reports and may be biased by the selection of patients in a rather small group. While Wu *et al*[58] found no significant association, Ye *et al*[59] determined the opposite: *H pylori* infection may protect from esophageal cancer (OR = 0.3). Thus, at this point, several works indicate that CagA has a protective effect on the development of esophageal cancer, however, no general conclusions can be made.

However, this association does not justify a general refusion of *H pylori* eradication because of two reasons. First, esophageal adenocarcinoma is far less common than gastric cancer. Second, the risk of developing adenocarcinoma of the esophagus is lowered by a factor 2-2.5 in the presence of *cagA*-positive *H pylori* infection whereas the risk for distal gastric cancer associated with *cagA*-positive strains is much higher (5-28x).

CONCLUSIONS

H pylori is a clear cut carcinogen. Infection with certain strain types in the presence of genetic polymorphisms leading to a heightened inflammatory response is associated with a dramatically increased relative risk to develop gastric cancer. Such developments can be foreseen by evaluating gastric biopsies, but also by determining serum pepsinogen levels as a marker of gastric atrophy. Endoscopy of the upper gastrointestinal tract (GI) should be performed before eradication to determine the status of gastric inflammation. Eradication of *H pylori* as prophylaxis of gastric adenocarcinoma is effective in early stages in which no severe histological changes have occurred. In countries with a high prevalence of infection and high cancer risks such as Japan or China, a general prophylactic eradication strategy seems to be beneficial especially in younger patients (< 50 years); nevertheless, the outcome of such long term prospective prophylactic studies needs to be evaluated. In Western countries with low prevalence rates and low cancer rates, a test-and-treat strategy is not cost-effective to prevent cancer; however, identification and treatment of risk groups seems rationale. Risk groups can be identified by evaluation of family history, presence of histological alterations in the gastric corpus, or by determining infection with *cagA*$^+$ strains as well as determining the status of genetic polymorphisms in the IL-1RN*2 gene.

REFERENCES

1 **Blaser MJ**. The biology of cag in the *Helicobacter pylori*-human interaction. *Gastroenterology* 2005; **128**: 1512-1515
2 **Blaser MJ**, Atherton JC. *Helicobacter pylori* persistence: biology and disease. *J Clin Invest* 2004; **113**: 321-333
3 **Blaser MJ**, Berg DE. *Helicobacter pylori* genetic diversity and risk of human disease. *J Clin Invest* 2001; **107**: 767-773
4 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789
5 **Sipponen P**. Gastric cancer: pathogenesis, risks, and prevention. *J Gastroenterol* 2002; **37** Suppl 13: 39-44
6 **Sipponen P**, Marshall BJ. Gastritis and gastric cancer. Western countries. *Gastroenterol Clin North Am* 2000; **29**: 579-592, v-vi
7 **Suerbaum S**, Michetti P. *Helicobacter pylori* infection. *N Engl J Med* 2002; **347**: 1175-1186
8 **Covacci A**, Telford JL, Del Giudice G, Parsonnet J, Rappuoli R. *Helicobacter pylori* virulence and genetic geography. *Science* 1999; **284**: 1328-1333
9 **Hohenberger P**, Gretschel S. Gastric cancer. *Lancet* 2003; **362**: 305-315
10 **Nomura A**, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med* 1991; **325**: 1132-1136
11 **Nomura AM**, Lee J, Stemmermann GN, Nomura RY, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* CagA seropositivity and gastric carcinoma risk in a Japanese American population. *J Infect Dis* 2002; **186**: 1138-1144

12 **Sendler A**, Böttcher K, Etter M, Siewert JR. [Gastric carcinoma]. *Internist* (Berl) 2000; **41**: 817-818, 821-826, 828-830
13 **Go MF**. Review article: natural history and epidemiology of *Helicobacter pylori* infection. *Aliment Pharmacol Ther* 2002; **16** Suppl 1: 3-15
14 **Inoue M**, Tsugane S. Epidemiology of gastric cancer in Japan. *Postgrad Med J* 2005; **81**: 419-424
15 **Miwa H**, Go MF, Sato N. H. pylori and gastric cancer: the Asian enigma. *Am J Gastroenterol* 2002; **97**: 1106-1112
16 **Go MF**. Diagnosis and Treatment of *Helicobacter pylori*. Curr *Treat Options Gastroenterol* 2005; **8**: 163-174
17 **Matsukura N**, Yamada S, Kato S, Tomtitchong P, Tajiri T, Miki M, Matsuhisa T, Yamada N. Genetic differences in interleukin-1 betapolymorphisms among four Asian populations: an analysis of the Asian paradox between H. pylori infection and gastric cancer incidence. *J Exp Clin Cancer Res* 2003; **22**: 47-55
18 **Brenner H**, Arndt V, Stegmaier C, Ziegler H, Rothenbacher D. Is *Helicobacter pylori* infection a necessary condition for noncardia gastric cancer? *Am J Epidemiol* 2004; **159**: 252-258
19 **Brenner H**, Arndt V, Stürmer T, Stegmaier C, Ziegler H, Dhom G. Individual and joint contribution of family history and *Helicobacter pylori* infection to the risk of gastric carcinoma. *Cancer* 2000; **88**: 274-279
20 **Brenner H**, Bode G, Boeing H. *Helicobacter pylori* infection among offspring of patients with stomach cancer. *Gastroenterology* 2000; **118**: 31-35
21 **Ekström AM**, Held M, Hansson LE, Engstrand L, Nyrén O. *Helicobacter pylori* in gastric cancer established by CagA immunoblot as a marker of past infection. *Gastroenterology* 2001; **121**: 784-791
22 **Huang JQ**, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-analysis of the relationship between cagA seropositivity and gastric cancer. *Gastroenterology* 2003; **125**: 1636-1644
23 **Azuma T**, Yamakawa A, Yamazaki S, Ohtani M, Ito Y, Muramatsu A, Suto H, Yamazaki Y, Keida Y, Higashi H, Hatakeyama M. Distinct diversity of the cag pathogenicity island among *Helicobacter pylori* strains in Japan. *J Clin Microbiol* 2004; **42**: 2508-2517
24 **Azuma T**, Yamazaki S, Yamakawa A, Ohtani M, Muramatsu A, Suto H, Ito Y, Dojo M, Yamazaki Y, Kuriyama M, Keida Y, Higashi H, Hatakeyama M. Association between diversity in the Src homology 2 domain--containing tyrosine phosphatase binding site of *Helicobacter pylori* CagA protein and gastric atrophy and cancer. *J Infect Dis* 2004; **189**: 820-827
25 **Hatakeyama M**. Oncogenic mechanisms of the *Helicobacter pylori* CagA protein. *Nat Rev Cancer* 2004; **4**: 688-694
26 **Aspholm-Hurtig M**, Dailide G, Lahmann M, Kalia A, Ilver D, Roche N, Vikström S, Sjöström R, Lindén S, Bäckström A, Lundberg C, Arnqvist A, Mahdavi J, Nilsson UJ, Velapatiño B, Gilman RH, Gerhard M, Alarcon T, López-Brea M, Nakazawa T, Fox JG, Correa P, Dominguez-Bello MG, Perez-Perez GI, Blaser MJ, Normark S, Carlstedt I, Oscarson S, Teneberg S, Berg DE, Borén T. Functional adaptation of BabA, the H. pylori ABO blood group antigen binding adhesin. *Science* 2004; **305**: 519-522
27 **Gerhard M**, Lehn N, Neumayer N, Borén T, Rad R, Schepp W, Miehlke S, Classen M, Prinz C. Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen-binding adhesin. *Proc Natl Acad Sci USA* 1999; **96**: 12778-12783
28 **Prinz C**, Schöniger M, Rad R, Becker I, Keiditsch E, Wagenpfeil S, Classen M, Rösch T, Schepp W, Gerhard M. Key importance of the *Helicobacter pylori* adherence factor blood group antigen binding adhesin during chronic gastric inflammation. *Cancer Res* 2001; **61**: 1903-1909
29 **Rad R**, Gerhard M, Lang R, Schöniger M, Rösch T, Schepp W, Becker I, Wagner H, Prinz C. The *Helicobacter pylori* blood group antigen-binding adhesin facilitates bacterial colonization and augments a nonspecific immune response. *J Immunol* 2002; **168**: 3033-3041
30 **Rad R**, Dossumbekova A, Neu B, Lang R, Bauer S, Saur D, Gerhard M, Prinz C. Cytokine gene polymorphisms influence mucosal cytokine expression, gastric inflammation, and host specific colonisation during *Helicobacter pylori* infection. *Gut* 2004; **53**: 1082-1089
31 **Rad R**, Prinz C, Neu B, Neuhofer M, Zeitner M, Voland P, Becker I, Schepp W, Gerhard M. Synergistic effect of *Helicobacter pylori* virulence factors and interleukin-1 polymorphisms for the development of severe histological changes in the gastric mucosa. *J Infect Dis* 2003; **188**: 272-281
32 **Bamford KB**, Fan X, Crowe SE, Leary JF, Gourley WK, Luthra GK, Brooks EG, Graham DY, Reyes VE, Ernst PB. Lymphocytes in the human gastric mucosa during *Helicobacter pylori* have a T helper cell 1 phenotype. *Gastroenterology* 1998; **114**: 482-492
33 **Ernst PB**, Gold BD. The disease spectrum of *Helicobacter pylori*: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. *Annu Rev Microbiol* 2000; **54**: 615-640
34 **Fan X**, Crowe SE, Behar S, Gunasena H, Ye G, Haeberle H, Van Houten N, Gourley WK, Ernst PB, Reyes VE. The effect of class II major histocompatibility complex expression on adherence of *Helicobacter pylori* and induction of apoptosis in gastric epithelial cells: a mechanism for T helper cell type 1-mediated damage. *J Exp Med* 1998; **187**: 1659-1669
35 **Wang J**, Brooks EG, Bamford KB, Denning TL, Pappo J, Ernst PB. Negative selection of T cells by *Helicobacter pylori* as a model for bacterial strain selection by immune evasion. *J Immunol* 2001; **167**: 926-934
36 **Fox JG**, Sheppard BJ, Dangler CA, Whary MT, Ihrig M, Wang TC. Germ-line p53-targeted disruption inhibits helicobacter-induced premalignant lesions and invasive gastric carcinoma through down-regulation of Th1 proinflammatory responses. *Cancer Res* 2002; **62**: 696-702
37 **Fox JG**, Beck P, Dangler CA, Whary MT, Wang TC, Shi HN, Nagler-Anderson C. Concurrent enteric helminth infection modulates inflammation and gastric immune responses and reduces helicobacter-induced gastric atrophy. *Nat Med* 2000; **6**: 536-542
38 **Bodger K**, Crabtree JE. *Helicobacter pylori* and gastric inflammation. *Br Med Bull* 1998; **54**: 139-150
39 **Crabtree JE**, Court M, Aboshkiwa MA, Jeremy AH, Dixon MF, Robinson PA. Gastric mucosal cytokine and epithelial cell responses to *Helicobacter pylori* infection in Mongolian gerbils. *J Pathol* 2004; **202**: 197-207
40 **Naumann M**, Crabtree JE. *Helicobacter pylori*-induced epithelial cell signalling in gastric carcinogenesis. *Trends Microbiol* 2004; **12**: 29-36
41 **Bodger K**, Bromelow K, Wyatt JI, Heatley RV. Interleukin 10 in *Helicobacter pylori* associated gastritis: immunohistochemical localisation and in vitro effects on cytokine secretion. *J Clin Pathol* 2001; **54**: 285-292
42 **Bodger K**, Wyatt JI, Heatley RV. Gastric mucosal secretion of interleukin-10: relations to histopathology, *Helicobacter pylori* status, and tumour necrosis factor-alpha secretion. *Gut* 1997; **40**: 739-744
43 **El-Omar EM**, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, Fraumeni JF Jr, Chow WH. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology* 2003; **124**: 1193-1201
44 **El-Omar EM**, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF Jr, Rabkin CS. The role of interleukin-1 polymorphisms in the pathogenesis of gastric cancer. *Nature* 2001; **412**: 99
45 **Garza-González E**, Bosques-Padilla FJ, El-Omar E, Hold G, Tijerina-Menchaca R, Maldonado-Garza HJ, Pérez-Pérez GI. Role of the polymorphic IL-1B, IL-1RN and TNF-A genes in distal gastric cancer in Mexico. *Int J Cancer* 2005; **114**: 237-241
46 **Macarthur M**, Hold GL, El-Omar EM. Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G515-G520
47 **Hwang IR**, Kodama T, Kikuchi S, Sakai K, Peterson LE, Graham DY, Yamaoka Y. Effect of interleukin 1

polymorphisms on gastric mucosal interleukin 1beta production in *Helicobacter pylori* infection. *Gastroenterology* 2002; **123**: 1793-1803

48 **Glas J**, Török HP, Schneider A, Brünnler G, Kopp R, Albert ED, Stolte M, Folwaczny C. Allele 2 of the interleukin-1 receptor antagonist gene is associated with early gastric cancer. *J Clin Oncol* 2004; **22**: 4746-4752

49 **Chang YW**, Jang JY, Kim NH, Lee JW, Lee HJ, Jung WW, Dong SH, Kim HJ, Kim BH, Lee JI, Chang R. Interleukin-1B (IL-1B) polymorphisms and gastric mucosal levels of IL-1beta cytokine in Korean patients with gastric cancer. *Int J Cancer* 2005; **114**: 465-471

50 **Yang J**, Hu Z, Xu Y, Shen J, Niu J, Hu X, Guo J, Wei Q, Wang X, Shen H. Interleukin-1B gene promoter variants are associated with an increased risk of gastric cancer in a Chinese population. *Cancer Lett* 2004; **215**: 191-198

51 **Chen A**, Li CN, Hsu PI, Lai KH, Tseng HH, Hsu PN, Lo GH, Lo CC, Lin CK, Hwang IR, Yamaoka Y, Chen HC. Risks of interleukin-1 genetic polymorphisms and *Helicobacter pylori* infection in the development of gastric cancer. *Aliment Pharmacol Ther* 2004; **20**: 203-211

52 **Zeng ZR**, Hu PJ, Hu S, Pang RP, Chen MH, Ng M, Sung JJ. Association of interleukin 1B gene polymorphism and gastric cancers in high and low prevalence regions in China. *Gut* 2003; **52**: 1684-1689

53 **Wong BC**, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, Ching CK, Chen JS. *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 2004; **291**: 187-194

54 **Zhou LY**, Shen ZY, Lin SR, Jin Z, Ding SG, Huang XB, Xia ZW, Liu JJ, Guo HL, William C. [Changes of gastric mucosa histopathology after *Helicobacter pylori* eradication]. *Zhonghua Neike Zazhi* 2003; **42**: 162-164

55 **Nomura AM**, Kolonel LN, Miki K, Stemmermann GN, Wilkens LR, Goodman MT, Perez-Perez GI, Blaser MJ. *Helicobacter pylori*, pepsinogen, and gastric adenocarcinoma in Hawaii. *J Infect Dis* 2005; **191**: 2075-2081

56 **Watabe H**, Mitsushima T, Yamaji Y, Okamoto M, Wada R, Kokubo T, Doi H, Yoshida H, Kawabe T, Omata M. Predicting the development of gastric cancer from combining *Helicobacter pylori* antibodies and serum pepsinogen status: a prospective endoscopic cohort study. *Gut* 2005; **54**: 764-768

57 **Chow WH**, Blaser MJ, Blot WJ, Gammon MD, Vaughan TL, Risch HA, Perez-Perez GI, Schoenberg JB, Stanford JL, Rotterdam H, West AB, Fraumeni JF Jr. An inverse relation between cagA+ strains of *Helicobacter pylori* infection and risk of esophageal and gastric cardia adenocarcinoma. *Cancer Res* 1998; **58**: 588-590

58 **Wu AH**, Crabtree JE, Bernstein L, Hawtin P, Cockburn M, Tseng CC, Forman D. Role of *Helicobacter pylori* CagA+ strains and risk of adenocarcinoma of the stomach and esophagus. *Int J Cancer* 2003; **103**: 815-821

59 **Ye W**, Held M, Lagergren J, Engstrand L, Blot WJ, McLaughlin JK, Nyrén O. *Helicobacter pylori* infection and gastric atrophy: risk of adenocarcinoma and squamous-cell carcinoma of the esophagus and adenocarcinoma of the gastric cardia. *J Natl Cancer Inst* 2004; **96**: 388-396

S- Editor Wang J **L- Editor** Rampone B **E- Editor** Liu WF

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5465-5472
World Journal of Gastroenterology ISSN 1007-9327

H pylori status and angiogenesis factors in human gastric carcinoma

Anita Mangia, Annalisa Chiriatti, Girolamo Ranieri, Ines Abbate, Maria Coviello, Giovanni Simone, Francesco Alfredo Zito, Severino Montemurro, Antonello Rucci, Alfredo Di Leo, Stefania Tommasi, Pasquale Berloco, Jian Ming Xu, Angelo Paradiso

Anita Mangia, Annalisa Chiriatti, Stefania Tommasi, Angelo Paradiso, Clinical Experimental Oncology Laboratory, National Cancer Institute, 70126 Bari, Italy
Girolamo Ranieri, Interventional Radiology Unit, National Cancer Institute, 70126 Bari, Italy
Ines Abbate, Maria Coviello, Chemical-Clinical-Microbiology and Immunology Laboratory Unit, National Cancer Institute, 70126 Bari, Italy
Giovanni Simone, Francesco Alfredo Zito, Histopathology Unit, National Cancer Institute, 70126 Bari, Italy
Severino Montemurro, Antonello Rucci, Gastroenterology Surgical Unit, National Cancer Institute, Bari, Italy
Alfredo Di Leo, Department of Emergency and Organ Transplantation, Section of Gastroenterology, University of Bari, Italy
Pasquale Berloco, Laboratory of Biochemistry, IRCCS "Saverio de Bellis", Castellana Grotte (BA), Italy
Jian Ming Xu, Department of Gastrointestinal Cancer, Beijing 307 Hospital Cancer Center, Beijing, China
Supported by grants from A I R C Project 2005, Italy
Correspondence to: Dr. Angelo Paradiso, MD, Clinical Experimental Oncology Laboratory, National Cancer Institute, Via Amendola, 209, Bari 70126, Italy. a.paradiso@oncologico.bari.it
Telephone: +39-80-5555561 Fax: +39-80-5555561
Received: 2004-12-21 Accepted: 2005-02-21

Abstract

AIM: To investigate *H pylori* expression in gastric cancer patients in relation to primary tumor angiogenic markers, such as microvessel density (MVD), thymidine phosphorylase (TP), vascular endothelial growth factor receptor-1 (VEGF-R1), p53 and circulating VEGF levels.

METHODS: Angiogenic markers were analyzed immunohistochemically in 56 primary gastric cancers. *H pylori* cytotoxin (*vac*A) and the cytotoxin-associated gene (*cag*A) amplification were evaluated using PCR assay. Serum *H pylori* IgG antibodies and serum/plasma circulating VEGF levels were detected in 39 and 38 patients by ELISA, respectively.

RESULTS: A total of 69% of patients were positive for circulating IgG antibodies against *H pylori*. *cag*A-positive *H pylori* strains were found in 41% of gastric patients. *vac*A was found in 50% of patients; s1 strains were more highly expressed among *vac*A-positive patients. The presence of the s1 strain was significantly associated with *cag*A (P = 0.0001). MVD was significantly correlated with both tumor VEGF expression (r = 0.361, P = 0.009) and serum VEGF levels (r = -0.347, P = 0.041). Conversely, neither VEGF-R1 expression nor MVD was related to p53 expression. However, *H pylori* was not related to any angiogenic markers except for the plasma VEGF level (P = 0.026).

CONCLUSION: *H pylori* antigen is related to higher plasma VEGF levels, but not to angiogenic characteristics. It can be hypothesized that the toxic effects of *H pylori* on angiogenesis occurs in early preclinical disease phase or in long-lasting aggressive infections, but only when high *H pylori* IgG levels are persistent.

Key words: *H pylori*; Gastric carcinoma; Angiogenesis

Mangia A, Chiriatti A, Ranieri G, Abbate I, Coviello M, Simone G, Zito FA, Montemurro S, Rucci A, Di Leo A, Tommasi S, Berloco P, Xu JM, Paradiso A. *H pylori* status and angiogenesis factors in human gastric carcinoma. *World J Gastroenterol* 2006; 12(34): 5465-5472

http://www.wjgnet.com/1007-9327/12/5465.asp

INTRODUCTION

H pylori infection is a well-known risk factor for the development of pre-neoplastic and neoplastic gastric mucosal alterations[1,2]. An increase in proliferative activity of gastric epithelial cells without a corresponding increase in apoptosis has been implicated in *H pylori* gastric carcinogenesis[3,4]. In addition, specific virulence determinants of *H pylori* strains can influence the outcome of the infection. Urease, vacuolating cytotoxin *vac*A, and the pathogenicity island (*cag* PAI) gene products are the main virulence factors of this organism involved in the development of gastric carcinoma. Thus, individuals infected with strains that express these virulence factors are prone to develop severe local inflammation which may induce the development of peptic ulcers and gastric cancers. Also, *H pylori* activity may be associated with virulence; in fact, urease activity may be an important colonization factor and exert a direct toxic effect upon intercellular junctions, resulting in alteration of gastric mucosal permeability[5]. The subsequent passage toward cancer is probably prompted by other factors, such as the onset of infection or other agents independent of *H pylori*.

Several studies have suggested that angiogenesis might also contribute to gastric tumorigenesis[6-8]. Angiogenesis is a complex multistep cascade modulated by positive soluble factors, such as the vascular endothelial growth factor (VEGF). The tumor neo-angiogenesis has been demonstrated in almost all solid tumors using various morphological techniques. The current method of angiogenesis quantification is the evaluation of CD34 antigen expression, a cell surface glycoprotein also present in the vascular endothelium permitting the study of intratumor endothelial cells[9]. The cellular receptor for VEGF, VEGF-R1 or Flt-1, is highly expressed in gastric carcinoma cells, suggesting that this pathway could influence tumor growth and metastasis through paracrine and autocrine mechanisms[10]. An additional tissue factor is thymidine phosphorylase (TP), an enzyme involved in pyrimidine nucleoside metabolism, which is identical to the platelet-derived endothelial cell growth factor and is endowed with angiogenic activities in various solid tumors[11] Furthermore, the *p53* oncosuppressor gene has been reported to be involved in inhibition of tumor vascularization by fostering unopposed angiopoietin-2 activity[12].

Recent publications have suggested that *H pylori* infection may regulate the angiogenesis and invasion of gastric carcinoma. In fact, *H pylori* influences *in vitro* angiogenesis-related gene expression; in particular, it has been demonstrated to up-regulate VEGF expression in gastric epithelial cells, an effect which appears to be related to *vac*A-expression[13,14]. Moreover, *H pylori* has been shown to up-regulate the expression of epidermal growth factor (EGF)-related growth factors and COX-2 in *in vitro* human gastric epithelial cells as well as in human gastric mucosa *in vivo*[15,16]. Lastly, its relationship with p53, which has been described as an angiogenesis-related factor, has been documented[17-20]. In spite of these evidences originating from *in vitro* studies, suggesting a relationship between pathophysiological roles for *H pylori* in the induction of tumor neo-angiogenesis**,** to our best of knowledge, no data are available in literature in patient series. Our hypothesis was that *H pylori*-related gastric cancer could involve different neo-angiogenic characteristics with respect to tumors without bacterial infection.

To verify the association between *H pylori* infection and different angiogenesis-related characteristics, 56 gastric cancer patients were studied for microvessel density (MVD), thymidine phosphorylase (TP), vascular endothelial growth factor-receptor (VEGF-R1) and p53 expressions in addition to circulating serum and plasma VEGF levels. *H pylori* was investigated at the molecular and at circulating blood levels.

MATERIALS AND METHODS

Patients

Fifty-six patients (37 men and 19 women; median age 64 years, range 42-83 years) with T_{1-4} N_{0-1} M_{0-1} gastric carcinoma were enrolled in this study. All patients had primary surgery for gastric cancer at National Cancer Institute of Bari. Primary tumor tissues were utilized for the immunohistochemical analysis of MVD, p53, VEGF-R1 and TP expressions.

Table 1 Clinicopathological features and distribution of *cagA*, *vacA* and IgG anti-*H pylori* in a series of 56 gastric cancer patients

Clinicopathological features	*n*
Sex	
Male	37
Female	19
Tumour category	
pT_{1-2-3}	28
pT_4	28
Location	
Antrum	23
Other	33
IgG anti *H pylori* (ELISA)	39
IgG - (≤ 7 KU/L)	12
IgG + (> 7 KU/L)	27
cagA (PCR)	56
cagA -	32
cagA +	23
NE	1
vacA (PCR)	56
NEG	28
s1m1	10
s2m2	5
s1m2	9
s1m1/s1m2[a]	1
NE	3

NEG: Negative; NE: Not evaluable; [a] Multiple genome.

Formalin-fixed and paraffin-embedded specimen of the primary tumor was selected by the pathologist for each patient on the basis of the quality of morphological preservation and neoplastic cellularity. In accordance with standardized sampling protocols, the sample was comprehensive both at the deeper portions of tumor, as well as the edges of the lesions. Five-micrometer thick sections were cut for immunohistochemical assay and for determination of *H pylori* status by means of polymerase chain reaction (PCR). A section contiguous to those selected for immunohistochemistry and DNA extraction was always stained with haematoxylin and eosin and confirmed by the pathologist as rich in neoplastic cellularity. Enzyme-linked immuno-sorbent assay (ELISA) for IgG antibodies against *H pylori* was performed on blood samples from 39 patients. Circulating VEGF levels were detected by ELISA in serum and plasma of 38 patients. The patients characteristics are shown in Table 1.

DNA extraction and PCR analysis

DNA extraction from paraffin-embedded specimens was performed using the method described by Lin *et al*[21]. Briefly, samples were incubated with a lysis buffer and proteinase K for 3 h at 55℃. Total DNA was extracted with phenol/chloroform, precipitated with acidic ethanol, and dissolved in sterile water.

Amplification of cagA and vacA

The extracted DNA was subjected to PCR for detection of *H pylori* genes, *cagA* and *vac*A. The *cag*A gene was amplified using the primers described elsewhere[22,23]. The *vac*A gene

was amplified using primers described by Atherton *et al*[24] which evaluate the mid region (*m*) and the region encoding for the signal peptide (s) of the gene. Four different PCR products were obtained: *s1* or *s2* from the *s* region, and *m1* and *m2* from the *m* region. PCR products were analyzed by electrophoresis on 20 g/L agarose gel. Positive and negative controls were examined with each batch of PCR.

Detection of anti-H pylori IgG

An enzyme-linked immuno-sorbent assay was used to detect *H pylori*-specific IgG serum antibodies (Anti-*H pylori* EIA Quant- COBAS2- Roche Diagnostics). The anti-*H pylori* IgG EIA is a second-generation two-step EIA for the detection of IgG antibodies to *H pylori* in human serum, based on a set of fast protein liquid chromatography-purified cell surface antigens, including the native urease enzyme[25]. According to the manufacturer, patients were considered positive for IgG against *H pylori* when IgG value was higher than 7 U/mL.

Immunohistochemistry

Serial sections of paraffin-embedded gastric tissue were deparaffinized and rehydrated. For antigen retrieval, the sections were microwaved at 500 W for 10 min in citrate buffer (pH 6) and endogenous peroxidase activity was blocked with 30 mL/L hydrogen peroxide solution. Adjacent slides were incubated with different monoclonal antibodies. The bound antibody was visualized using a biotinylated secondary antibody, avidin-biotin peroxidase complex, and 3-amino-9-ethylcarbazole (Ultra Vision Detection System anti-Polyvalent, HRP/DAB, Lab Vision Corporation). For negative control sections, primary antibody was replaced with phosphate-buffered saline and processed in the same manner. Gastric carcinomas known to express high levels of CD34, p53, TP and VEGF-R1 proteins were used as positive controls.

Anti-CD34 (QB–END/10, Novocastra Laboratories Ltd) was diluted at 1:50 for 1 h at room temperature as a pan-endothelial marker for MVD analysis. The modified Weidner's method was utilized for the evaluation of MVD according to CD34 endothelial cell immunostaining[26]. For the microvessel counting, positive stainings for MVD, in five most highly vascularized areas ('hot spots') in each slide, were counted in 400 × fields with an image analysis system (Quantimet 500 Leica; 0.19 mm^2/field) and MVD was expressed as the average of the microvessel count in these areas[27]. Any EC or endothelial cluster positive for CD34 (brown yellow staining) was considered to be a single countable microvessel. Sclerotic areas, both hypocellular and necrotic, within the tumor were not considered for vessel evaluation.

Anti-p53 monoclonal antibodies (PAb 1801, Neo-Markers), grown against human p53 and recognizing wild-type and mutant forms of the p53 protein, were diluted at 1:150 for 1 h at room temperature. Tumor cells expressing p53 immunoreactivity were quantified by evaluating a total of 1000 neoplastic cells in random fields from representative areas. Exclusive nuclear staining was scored as positive. The immunoreactive cells were expressed as percentages[28]. Anti-TP monoclonal antibodies (P-GF.44C Neo-Markers), recognizing full length human TP protein, were diluted at 1:100 for 1 h at room temperature. TP positivity was determined at 400 × fields with the image analysis system and was evaluated on the basis of percentage of stained epithelial tumor cells. Tumor cells with moderate or strong staining intensity were counted. TP expression in macrophages was considered an internal positive control. The polyclonal antibody anti-VEGF-R1 (Flt-1 polyclonal rabbit antibody, Santa Cruz Biotechnology Inc.), recognizing the carboxyl terminus of the receptor for VEGF, VEGF-R1 or the Flt-1 protein of human origin, was used at a dilution of 1:100 for 1 h at room temperature. VEGF-R1 positivity was scored as cytoplasmic immunostaining using an image analysis system (Quantimet 500 Leica). Immunoreactivity was expressed as the percentage ratio between the area of immuno-positive tumor cells and the entire area of invasive neoplastic tissue.

The laboratory, where the immunohistochemical analyses were performed, participated to Quality Control programs managed by INQAT[29].

Circulating VEGF detection

Blood samples were collected before surgery. Venous blood was dispensed into a serum separator tube (Becton Dickinson Vacutainer Systems) for serum obtainment, and into sodium citrate, theophylline, adenosine, dipyridamole (CTAD) tubes for plasma (Becton Dickinson Hemogard Vacutainer Systems).

Circulating VEGF levels were examined in plasma and serum using the Quantikine Human VEGF-enzyme-linked immuno-sorbent assay (ELISA, R&D System Inc.) which recognizes VEGF165. According to the manufacturer, the minimum detectable dose of VEGF is typically less than 9.0 ng/L. Values below 9.0 ng/L were equal to zero. VEGF levels in plasma and serum were analyzed, as we previously demonstrated that the two determinations provide alternative and additional information on circulating VEGF, also in relation to the role played by the activation and quantity of platelets in VEGF release[30].

Statistical analysis

The associations between MVD, TP, VEGF-R1 and p53 expression, markers of *H pylori* status and histological diagnosis were evaluated using the Chi-square test. A correlation analysis among the aforementioned biomarkers, considered as continuous variables, was performed by Pearson's correlation coefficient (*r*). In the statistical analysis, *vac*A genotypes were classified into two subgroups: "cytotoxic strains" which included s1m1, s1m2 and s1m1/s1m2 strains and "others" which included negative and s2m2. Patients with s1m1/s1m2 strains were infected by multiple genotypes. Backwards stepwise logistic regression analysis was used to estimate the independent association of any biological markers with *H pylori* characteristics. Statistical analysis was carried out using the software SPSS for windows, release 9.0.

RESULTS

H pylori status

Cytotoxin-associated gene (*cag*A)-positive *H pylori* strains

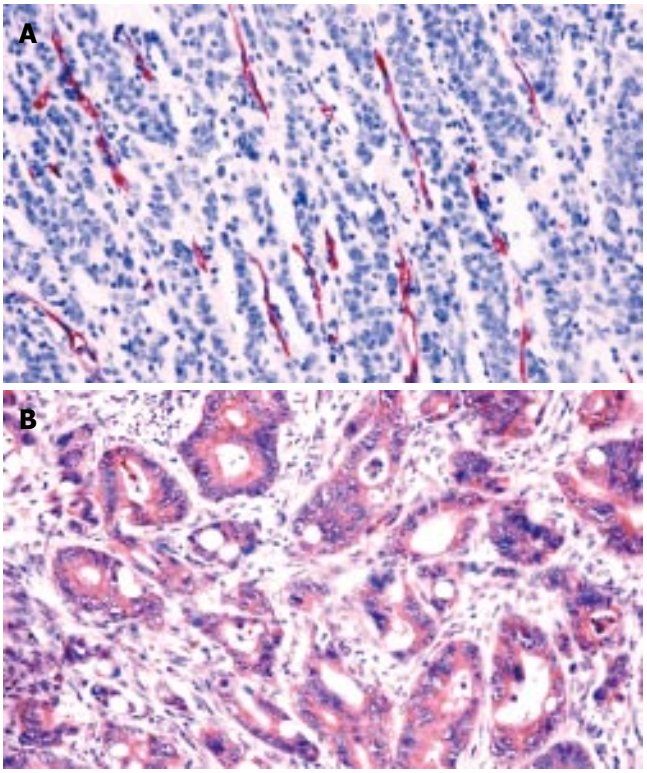


Figure 1 Immunohistochemical assay for detection of microvessel density (**A**) and VEGF-R1 protein expression (**B**) in gastric cancer. VEGF-R1 is stained in the cytoplasm of cancer cells.

were found in 41% of gastric patients. *vac*A was found in 50% of patients; s1 strains were more highly expressed among *vac*A-positive patients. Moreover, a single patient was found to be infected with different *H pylori* strains (multiple genomes). The results are summarized in Table 1. The presence of the s1 strain was significantly associated with c*agA* (P = 0.0001). Only one patient infected with the s2 strain showed *cag*A-positivity, while 26 (81%) patients were negative for both *cag*A and *vac*A. A total of 69% of patients were positive for circulating IgG antibodies against *H pylori*, with a mean and median value of 77 U/mL (range 1-476 U/mL) and 15 U/mL, respectively.

Immunohistochemical analysis

TP immunoreactivity was observed in normal epithelial cells, malignant epithelial cells, macrophages and endothelial cells. The usual pattern of positive staining was both cytoplasmic and nuclear. A mean of 5% (range 0%-80%) of cells showed TP positivity.

p53 expression was generally confined to neoplastic tissues, while the normal mucosa was rarely stained. A mean of 3% (range 0%-50%) of cells showed p53 positivity.

CD34 immunostaining was detected in the endothelial cells, especially in the area surrounding the tumor (Figure 1A). In the 'hot spot' tumor area, a mean of 39 vessels (range 0-100 vessels) was found with only 2% of cases not demonstrating any microvessels. VEGF-R1 immunostaining was mainly localized at the membrane and cytoplasm of epithelial and endothelial cells (Figure 1B). By counting only the epithelial component, a mean of 24% (range 0%-100%) of VEGF-R1-immunostained cells was found.

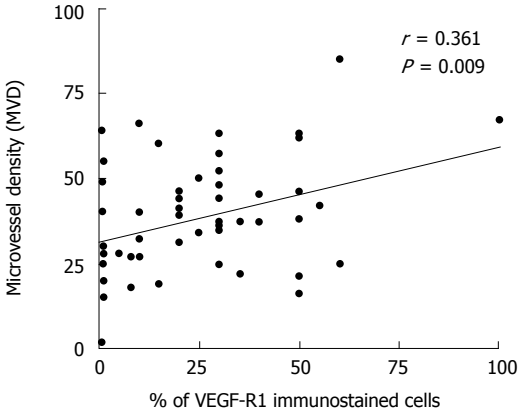


Figure 2 Correlation between percentage of VEGF-R1-immunoreactive cells and microvessel density (MVD) within each tumor.

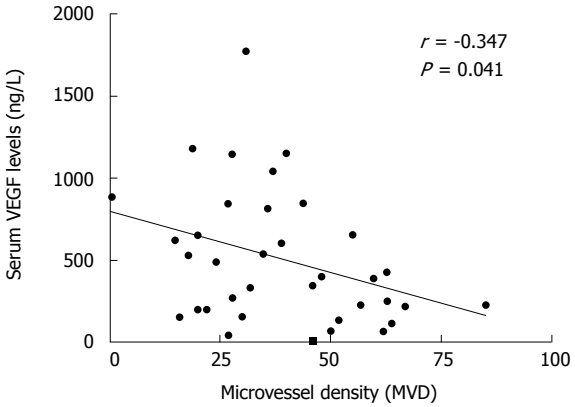


Figure 3 Correlation between serum VEGF levels and microvessel density (MVD) within each tumor.

About 80% of tumors demonstrated VEGF-R1 expression.

Regarding the relationship among the different angiogenic characteristics, only MVD was significantly correlated with both tumor VEGF expression (r = 0.361, P = 0.009; Figure 2) and serum VEGF levels (r = -0.347, P = 0.041; Figure 3). Conversely, neither VEGF-R1 expression nor MVD was related to p53 expression.

Table 2 shows the association between the markers of *H pylori* status and angiogenic factors. There was no significant association between markers of *H pylori* status, *cag*A, *vac*A and angiogenic biomarker expression. When the correlation between angiogenesis related-markers and IgG status was analysed a significant correlation between IgG status and plasma VEGF levels was observed (P = 0.026). The lack of association between *H pylori* characteristics and biomarkers was also confirmed with multivariate logistic regression analysis.

Angiogenic marker expression and markers of *H pylori* status were analyzed with respect to clinico-pathological features. Plasma VEGF levels and tumor TP expression were both significantly associated with tumor size (P = 0.030 and P = 0.035, respectively; Table 3). Regarding plasma VEGF levels in particular, T_4 tumors showed a significantly smaller percentage of low IgG cases as compared with T_{1-3} tumors (31% *vs* 74%; $P < 0.03$).

Table 2 ***cag*** **A,** ***vac*** **A, IgG anti-*H pylori* and angiogenic factors in gastric cancer patients**

	IgG anti *H pylori*		*cag* A		*vac* A	
	n (%) With IgG- (≤ 7 U/mL)	*n* (%) With IgG+ (> 7 U/mL)	*n* (%) Negative for *cag*A	*n* (%) Positive for *cag*A	*n* (%) Cytotoxic strains (s1; m1/2)	*n* (%) Others (absent; s2m2)
TP expression[1]						
0[2]	9 (31)	20 (69)	24 (57)	18 (43)	24 (60)	16 (40)
> 0	2 (25)	6 (75)	8 (80)	2 (10)	8 (80)	2 (20)
p53 expression[1]						
0[2]	8 (25)	24 (75)	27 (60)	18 (40)	28 (65)	15 (35)
> 0	3 (60)	2 (40)	5 (63)	3 (37)	4 (50)	4 (50)
MVD (CD34)[1]						
≤ 37[2]	7 (32)	15 (68)	16 (59)	11 (41)	15 (58)	11 (42)
> 37	3 (21)	11 (79)	16 (64)	9 (36)	17 (71)	7 (29)
VEGF-R1 expression[1]						
≤ 20[2]	6 (29)	15 (71)	16 (59)	11 (41)	14 (54)	12 (46)
> 20	5 (31)	11 (69)	15 (58)	11 (42)	17 (68)	8 (32)
pVEGF levels						
≤ 26[2]	9 (50)[a]	9 (50)[a]	10 (53)	9 (47)	11 (61)	7 (39)
> 26	2 (13)[a]	13 (87)[a]	9 (60)	6 (40)	9 (60)	6 (40)
sVEGF levels						
≤ 432[2]	5 (29)	12 (71)	11 (61)	7 (49)	12 (71)	5 (29)
> 432	6 (32)	13 (68)	9 (47)	10 (53)	9 (47)	10 (53)

sVEGF: Serum VEGF; pVEGF: Plasma VEGF; [1] % of immunostained cells; [2] Cut-off median value of the series; [a]$P = 0.026$.

Table 3 Association between angiogenic characteristics, markers of *H pylori* status and clinicopathological features

Biomarkers	Tumor stage			M status		
	T_{1-2-3} (*n* = 28)	T_4 (*n* = 28)	*P*	M_0 (*n* = 38)	M_1 (*n* = 18)	*P*
cagA negative (*n* = 32)	54	63	NS	58	59	NS
vacA no cytotoxic strains (*n* = 33)	62	63	NS	58	71	NS
TP negative (*n* = 42)	93	65	0.035	83	71	NS
p53 negative (*n* = 46)	78	93	NS	78	100	0.038
low MVD values (*n* = 27)	56	46	NS	54	44	NS
Low VEGF-R1 expression (*n* = 27)	56	44	NS	51	47	NS
High IgG levels (*n* = 27)	65	74	NS	77	54	NS
Low pVEGF levels (*n* = 19)	74	31	0.03	52	58	NS
Low sVEGF levels (*n* = 19)	35	67	NS	50	50	NS

NS: Non-significant.

Lastly, p53 expression was significantly associated with metastatic status ($P = 0.038$), as 100% of patients with metastatic disease did not express p53. No association was found between cytohistological tumor grading or *H pylori* infection site and angiogenesis-related markers and *H pylori* characteristics.

DISCUSSION

The role of *H pylori* in gastric cancerogenesis has been extensively investigated; conversely, information is lacking regarding the biological impact of *H pylori* on the progression of gastric cancer. Several factors emphasize the importance that various *H pylori* components can have their roles on the development of pre-neoplastic and neoplastic alterations of the gastric mucosa. Specific virulence factors produced by the bacterium, such as the vacuolating cytotoxin (vacA) or the cytotoxin-associated protein (cagA), contribute to gastroduodenal mucosal injury and impair the healing process of the damaged mucosa[31,32]. In addition, the host response to the infection and the presence of environmental factors are thought to be involved in the pathogenesis of *H pylori*-related gastroduodenal disease[33,34]. The vacuolating toxin (vacA) is believed to be a major determinant of *H pylori*-associated gastric disease[35,36]. The vacuolating cytotoxin gene A (*vacA*), which encodes the v*acA* protein, is present in all *H pylori* strains, but its encoded products are associated both with and without *in vitro* vacuolating activity[37]. It has been suggested that the *vac*A s1a genotype is closely associated with

high cytotoxin production, while the *vac*A s2 allele can demonstrate a negative *in vitro* association with cytotoxin activity. The presence of these virulence factors can be used to identify patients at risk to develop gastric cancer; in fact, patients with neoplastic transformation of the gastric mucosa are more likely to be infected by the *cag*A+ strain[19,38,39]. However, conflicting results regarding the association between these virulence factors and clinical outcome of gastric cancer are found in the literature[36,40-43].

p53 mutations and the genotypic characterization of *H pylori* have also been thoroughly studied to identify possible links between *H pylori* infection and p53 alterations without reaching definitive conclusions. Alterations of the *p53* gene and/or its abnormal protein accumulation have both been described during the later stage of gastric carcinogenesis[44,45] and in precancerous gastric lesions[46]. As *cag*A+ *H pylori* strains induce particularly severe inflammation in the gastric mucosa[41,47]. it has been hypothesized that gastric tumors from subjects infected with *cag*A+ *H pylori* might have a higher prevalence of p53 mutation than tumors from non-infected subjects.

H pylori infection may also regulate the angiogenesis and invasion of gastric carcinomas[13,14], but whether *H pylori* exerts its effects to induce neovascularization early in the development of gastric pre-neoplastic lesions or late in clinical phases of the disease is still unknown. However, it is clear that *H pylori* infection can increase the expression of the platelet-derived endothelial cell growth factor by infiltrating interstitial cells in pre-malignant lesions, such as intestinal metaplasia, thereby assisting in creating a favourable environment for tumor development[48]. Furthermore, it has been demonstrated that *H pylori* is able to up-regulate VEGF expression in gastric epithelial cells determining effects related to vacA-expression.

Recently, for the first time, Caputo *et al*[13] showed that *H pylori* up-regulated VEGF expression in gastric mucosa cells *in vitro* and that this effect was strictly *vac*A-dependent; and, interestingly, this result was not observed when using an isogenic mutant specifically lacking *vac*A. Moreover, *in vitro* and *in vivo* up-regulation of a number of EGF-related growth factors have also been reported[15,16,49].

In our sufficiently large series of gastric cancer patients, a percentage of *H pylori* infection was demonstrated, either as IgG-circulating level or as *cag*A/*vac*A DNA, which is in agreement with the previous studies[50,51]. In addition, the neo-angiogenesis characteristics reported did not significantly differ from those of other series of gastric cancers[7,52,53]. It is also possible to verify a clear relationship between p53, TP, VEGF and the clinicopathological characteristics considered in our series, further stressing the impact that angiogenesis has on tumor aggressiveness[52,53]. In fact, TP expression and plasma VEGF levels were both associated with tumor size, while p53 expression was associated with metastatic status.

However, when addressing the main objective of our study, it is possible to demonstrate an association only between higher levels of plasma VEGF and high levels of IgG (Table 3). Conversely, *H pylori*-infected tumors did not show p53, MVD, TP, VEGF-R1 characteristics which obviously differed from those without presence of *H pylori* infection. These results only partially agree with previous data[17,54] which, however, were all obtained from experimental *in vitro* models, in some cases referring to mRNA analyses utilizing only quantitative molecular or immuno-enzymatic approaches[14], therefore not exploiting morphological antigen tissue distribution. Furthermore, the increase of gene expression induced by the co-culture of gastric tumor cells with *H pylori* has been reported to be generally modest with more evident positive modulation for angiogenic factors not investigated in the present study, such as interleukin-8[14]. Caputo *et al*[13] also recently suggested that *vac*A-induced up-regulation of VEGF expression could depend on the functionality of epidermal growth factor receptor (EGFR)-, mitogen-activated protein kinase (MAPK)- and COX-2-mediated pathways, the biological targets which are largely heterogeneous in human gastric cancer. In conclusion, our study seems to suggest that the relationship between the *H pylori* toxic effect and angiogenic factors demonstrated *in vitro* could be influenced in human gastric tumor tissues by other key biological factors not considered in the present study.

A last comment regarding IgG and VEGF association concerns the blood of gastric cancer patients. The level of IgG antibodies has been suggested to be useful, not for diagnosis of infection, but for monitoring the outcome of *H pylori* infection over time, and specifically the efficacy of therapies aiming to eradicate *H pylori* infection. Thus, an elevated serum level before primary surgery for gastric cancer could be a signal of long-lasting and probably *H pylori* infection resistant to antimicrobial therapy[55].

The results of our study indicate that, from the angiogenic point of view, *H pylori*-related gastric cancers do not differ from those in which exposure to the bacterium cannot be demonstrated. Different explanations for these findings can be proposed: an angiogenetic relationship, if any, could be only induced by a long-lasting *H pylori* infection demonstrated by high IgG levels in the plasma; an alternative hypothesis might regard the ability of *H pylori* to modulate angiogenesis only during early phase of disease genesis and progression to be then lost during the clinically evident disease phases. This hypothesis would concord with the presumed role that angiogenesis plays, especially during the extremely early phases of cancer[9,17,56].

ACKNOWLEDGMENTS

We thank Mr. Baldassarre Stea for statistical analysis, Mr. Cesare Salvatore for technical support and Mrs. Paulene Maselli-Campagna for her help in the elaboration of the manuscript.

REFERENCES

1 **Marshall BJ**. Helicobacter pylori. *Am J Gastroenterol* 1994; **89**: S116-S128
2 **Lambert JR**, Lin SK, Aranda-Michel J. *Helicobacter pylori. Scand J Gastroenterol Suppl* 1995; **208**: 33-46
3 **Linsalata M**, Russo F, Notarnicola M, Berloco P, Di Leo A. Polyamine profile in human gastric mucosa infected by Helicobacter pylori. *Ital J Gastroenterol Hepatol* 1998; **30**: 484-489
4 **Ierardi E**, Francavilla A, Balzano T, Traversa A, Principi M, Monno RA, Amoruso A, Ingrosso M, Pisani A, Panella C. Ef-

fect of Helicobacter pylori eradication on gastric epithelial proliferation. Relationship with ras oncogene p21 expression. *Ital J Gastroenterol Hepatol* 1997; **29**: 214-219

5 **Dunn BE**, Sung CC, Taylor NS, Fox JG. Purification and characterization of Helicobacter mustelae urease. *Infect Immun* 1991; **59**: 3343-3345

6 **Giatromanolaki A**, Koukourakis MI, Stathopoulos GP, Kapsoritakis A, Paspatis G, Kakolyris S, Sivridis E, Georgoulias V, Harris AL, Gatter KC. Angiogenic interactions of vascular endothelial growth factor, of thymidine phosphorylase, and of p53 protein expression in locally advanced gastric cancer. *Oncol Res* 2000; **12**: 33-41

7 **Feng CW**, Wang LD, Jiao LH, Liu B, Zheng S, Xie XJ. Expression of p53, inducible nitric oxide synthase and vascular endothelial growth factor in gastric precancerous and cancerous lesions: correlation with clinical features. *BMC Cancer* 2002; **2**: 8

8 **Tuccillo C**, Cuomo A, Rocco A, Martinelli E, Staibano S, Mascolo M, Gravina AG, Nardone G, Ricci V, Ciardiello F, Del Vecchio Blanco C, Romano M. Vascular endothelial growth factor and neo-angiogenesis in H pylori gastritis in humans. *J Pathol* 2005; **207**: 277-284

9 **Zhang H**, Wu J, Meng L, Shou CC. Expression of vascular endothelial growth factor and its receptors KDR and Flt-1 in gastric cancer cells. *World J Gastroenterol* 2002; **8**: 994-998

10 **Tenderenda M**, Rutkowski P, Jesionek-Kupnicka D, Kubiak R. Expression of CD34 in gastric cancer and its correlation with histology, stage, proliferation activity, p53 expression and apoptotic index. *Pathol Oncol Res* 2001; **7**: 129-134

11 **Shimaoka S**, Matsushita S, Nitanda T, Matsuda A, Nioh T, Suenaga T, Nishimata Y, Akiba S, Akiyama S, Nishimata H. The role of thymidine phosphorylase expression in the invasiveness of gastric carcinoma. *Cancer* 2000; **88**: 2220-2227

12 **Tse V**, Yung Y, Santarelli JG, Juan D, Hsiao M, Haas M, Harsh G 4th, Silverberg G. Effects of tumor suppressor gene (p53) on brain tumor angiogenesis and expression of angiogenic modulators. *Anticancer Res* 2004; **24**: 1-10

13 **Caputo R**, Tuccillo C, Manzo BA, Zarrilli R, Tortora G, Blanco Cdel V, Ricci V, Ciardiello F, Romano M. Helicobacter pylori VacA toxin up-regulates vascular endothelial growth factor expression in MKN 28 gastric cells through an epidermal growth factor receptor-, cyclooxygenase-2-dependent mechanism. *Clin Cancer Res* 2003; **9**: 2015-2021

14 **Kitadai Y**, Sasaki A, Ito M, Tanaka S, Oue N, Yasui W, Aihara M, Imagawa K, Haruma K, Chayama K. Helicobacter pylori infection influences expression of genes related to angiogenesis and invasion in human gastric carcinoma cells. *Biochem Biophys Res Commun* 2003; **311**: 809-814

15 **Romano M**, Ricci V, Di Popolo A, Sommi P, Del Vecchio Blanco C, Bruni CB, Ventura U, Cover TL, Blaser MJ, Coffey RJ, Zarrilli R. Helicobacter pylori upregulates expression of epidermal growth factor-related peptides, but inhibits their proliferative effect in MKN 28 gastric mucosal cells. *J Clin Invest* 1998; **101**: 1604-1613

16 **Tuccillo C**, Manzo BA, Nardone G, D'Argenio G, Rocco A, Di Popolo A, Della VN, Staibano S, De Rosa G, Ricci V, Del Vecchio BC, Zarrilli R, Romano M. Up-regulation of heparin binding epidermal growth factor-like growth factor and amphiregulin expression in Helicobacter pylori-infected human gastric mucosa. *Dig Liver Dis* 2002; **34**: 498-505

17 **Berloco P**, Russo F, Cariola F, Gentile M, Giorgio P, Caruso ML, Valentini AM, Di Matteo G, Di Leo A. Low presence of p53 abnormalities in H pylori-infected gastric mucosa and in gastric adenocarcinoma. *J Gastroenterol* 2003; **38**: 28-36

18 **Wu MS**, Chen SY, Shun CT, Lee WJ, Wang HP, Wang TH, Chen CJ, Lin JT. Increased prevalence of Helicobacter pylori infection among patients affected with intestinal-type gastric cancer at non-cardiac locations. *J Gastroenterol Hepatol* 1997; **12**: 425-428

19 **Deguchi R**, Takagi A, Kawata H, Inoko H, Miwa T. Association between CagA+ Helicobacter pylori infection and p53, bax and transforming growth factor-beta-RII gene mutations in gastric cancer patients. *Int J Cancer* 2001; **91**: 481-485

20 Teh M, Tan KB, Seet BL, Yeoh KG. Study of *p53* immunostaining in the gastric epithelium of cagA-positive and cagA-negative Helicobacter pylori gastritis. *Cancer* 2002; **95**: 499-505

21 **Lin TT**, Yeh CT, Yang E, Chen PC. Detection of Helicobacter pylori by polymerase chain reaction assay using gastric biopsy specimens taken for CLOtest. *J Gastroenterol* 1996; **31**: 329-332

22 **Clayton CL**, Kleanthous H, Coates PJ, Morgan DD, Tabaqchali S. Sensitive detection of Helicobacter pylori by using polymerase chain reaction. *J Clin Microbiol* 1992; **30**: 192-200

23 **Tummuru MK**, Cover TL, Blaser MJ. Cloning and expression of a high-molecular-mass major antigen of Helicobacter pylori: evidence of linkage to cytotoxin production. *Infect Immun* 1993; **61**: 1799-1809

24 **Atherton JC**, Cao P, Peek RM Jr, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995; **270**: 17771-17777

25 **Goossens H**, Glupczynski Y, Burette A, Van den Borre C, DePrez C, Bodenmann J, Keller A, Butzler JP. Evaluation of a commercially available complement fixation test for diagnosis of Helicobacter pylori infection and for follow-up after antimicrobial therapy. *J Clin Microbiol* 1992; **30**: 3230-3233

26 **Vermeulen PB**, Gasparini G, Fox SB, Toi M, Martin L, McCulloch P, Pezzella F, Viale G, Weidner N, Harris AL, Dirix LY. Quantification of angiogenesis in solid human tumours: an international consensus on the methodology and criteria of evaluation. *Eur J Cancer* 1996; **32**A: 2474-2484

27 **Ranieri G**, Labriola A, Achille G, Florio G, Zito AF, Grammatica L, Paradiso A. Microvessel density, mast cell density and thymidine phosphorylase expression in oral squamous carcinoma. *Int J Oncol* 2002; **21**: 1317-1323

28 **Paradiso A**, Ranieri G, Simone G, Silvestris N, Costa A, De Lena M, Leone A, Vallejo C, Lacava J. mdm2-p53 Interaction: lack of correlation with the response to 5-fluorouracil in advanced colorectal cancer. *Oncology* 2002; **62**: 278-285

29 **Paradiso A**, Volpe S, Iacobacci A, Marubini E, Verderio P, Costa A, Daidone MG, Marchetti A, Mottolese M, Amadori D, De Paola F, Saragoni L, Medri L, Nenci I, Querzoli P, Gion M, Dittadi R, Plebani M, Orlando C, Bevilacqua G, Silvestrini R. Quality control for biomarker determination in oncology: the experience of the Italian Network for Quality Assessment of Tumor Biomarkers (INQAT). *Int J Biol Markers* 2002; **17**: 201-214

30 **Ranieri G**, Coviello M, Chiriatti A, Stea B, Montemurro S, Quaranta M, Dittadi R, Paradiso A. Vascular endothelial growth factor assessment in different blood fractions of gastrointestinal cancer patients and healthy controls. *Oncol Rep* 2004; **11**: 435-439

31 **Covacci A**, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, Massone A, Papini E, Xiang Z, Figura N. Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci USA* 1993; **90**: 5791-5795

32 **Cover TL**, Blaser MJ. Purification and characterization of the vacuolating toxin from Helicobacter pylori. *J Biol Chem* 1992; **267**: 10570-10575

33 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789

34 **Montecucco C**, Rappuoli R. Living dangerously: how Helicobacter pylori survives in the human stomach. *Nat Rev Mol Cell Biol* 2001; **2**: 457-466

35 **Cover TL**, Tummuru MK, Cao P, Thompson SA, Blaser MJ. Divergence of genetic sequences for the vacuolating cytotoxin among Helicobacter pylori strains. *J Biol Chem* 1994; **269**: 10566-10573

36 **Miehlke S**, Kirsch C, Agha-Amiri K, Günther T, Lehn N, Malfertheiner P, Stolte M, Ehninger G, Bayerdörffer E. The Helicobacter pylori vacA s1, m1 genotype and cagA is associated with gastric carcinoma in Germany. *Int J Cancer* 2000; **87**: 322-327

37 **Atherton JC**, Cover TL, Papini E, Telford JL. Vacuolating

Cytotoxin. In: Mobley HLT, Mendz GL, Hazell SL eds. *Helicobacter pylori*. Physiology and Genetics, 2001: 97-109

38 **Magalhães PP**, Queiroz DM, Camargo AA, Simpson AJ. CagA status of Helicobacter pylori infection and p53 gene mutations in gastric adenocarcinoma. *Carcinogenesis* 2003; **24**: 145; author reply 147

39 **Shibata A**, Parsonnet J, Longacre TA, Garcia MI, Puligandla B, Davis RE, Vogelman JH, Orentreich N, Habel LA. CagA status of Helicobacter pylori infection and p53 gene mutations in gastric adenocarcinoma. *Carcinogenesis* 2002; **23**: 419-424

40 **van Doorn LJ**, Figueiredo C, Sanna R, Plaisier A, Schneeberger P, de Boer W, Quint W. Clinical relevance of the cagA, vacA, and iceA status of Helicobacter pylori. *Gastroenterology* 1998; **115**: 58-66

41 **Yamaoka Y**, Kodama T, Graham DY, Kashima K. Comparison of four serological tests to determine the CagA or VacA status of Helicobacter pylori strains. *J Clin Microbiol* 1998; **36**: 3433-3434

42 **Blaser MJ**, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995; **55**: 2111-2115

43 **Rudi J**, Kolb C, Maiwald M, Zuna I, von Herbay A, Galle PR, Stremmel W. Serum antibodies against Helicobacter pylori proteins VacA and CagA are associated with increased risk for gastric adenocarcinoma. *Dig Dis Sci* 1997; **42**: 1652-1659

44 **Yamada Y**, Yoshida T, Hayashi K, Sekiya T, Yokota J, Hirohashi S, Nakatani K, Nakano H, Sugimura T, Terada M. p53 gene mutations in gastric cancer metastases and in gastric cancer cell lines derived from metastases. *Cancer Res* 1991; **51**: 5800-5805

45 **Tamura G**, Kihana T, Nomura K, Terada M, Sugimura T, Hirohashi S. Detection of frequent p53 gene mutations in primary gastric cancer by cell sorting and polymerase chain reaction single-strand conformation polymorphism analysis. *Cancer Res* 1991; **51**: 3056-3058

46 **Lauwers GY**, Wahl SJ, Melamed J, Rojas-Corona RR. *p53* expression in precancerous gastric lesions: an immunohistochemical study of PAb 1801 monoclonal antibody on adenomatous and hyperplastic gastric polyps. *Am J Gastroenterol* 1993; **88**: 1916-1919

47 **Kuipers EJ**, Pérez-Pérez GI, Meuwissen SG, Blaser MJ. Helicobacter pylori and atrophic gastritis: importance of the cagA status. *J Natl Cancer Inst* 1995; **87**: 1777-1780

48 **Franceschi F**, Genta RM, Gasbarrini A, Gentiloni Silveri N, Gasbarrini G, Sepulveda AR. Helicobacter pylori infection and expression of the angiogenic factor platelet-derived endothelial cell growth factor by pre-neoplastic gastric mucosal lesions and gastric carcinoma. *Dig Liver Dis* 2002; **34**: 621-625

49 **Romano M**, Ricci V, Memoli A, Tuccillo C, Di Popolo A, Sommi P, Acquaviva AM, Del Vecchio Blanco C, Bruni CB, Zarrilli R. Helicobacter pylori up-regulates cyclooxygenase-2 mRNA expression and prostaglandin E2 synthesis in MKN 28 gastric mucosal cells in vitro. *J Biol Chem* 1998; **273**: 28560-28563

50 **Iaquinto G**, Todisco A, Giardullo N, D'Onofrio V, Pasquale L, De Luca A, Andriulli A, Perri F, Rega C, De Chiara G, Landi M, Taccone W, Leandro G, Figura N. Antibody response to Helicobacter pylori CagA and heat-shock proteins in determining the risk of gastric cancer development. *Dig Liver Dis* 2000; **32**: 378-383

51 **Enroth H**, Kraaz W, Engstrand L, Nyrén O, Rohan T. Helicobacter pylori strain types and risk of gastric cancer: a case-control study. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 981-985

52 **Takahashi Y**, Cleary KR, Mai M, Kitadai Y, Bucana CD, Ellis LM. Significance of vessel count and vascular endothelial growth factor and its receptor (KDR) in intestinal-type gastric cancer. *Clin Cancer Res* 1996; **2**: 1679-1684

53 **Huang SP**, Wu MS, Shun CT, Wang HP, Lin JT. Tumor angiogenesis increases with nuclear p53 accumulation in gastric carcinoma. *Hepatogastroenterology* 2002; **49**: 1453-1456

54 **Ławniczak M,** Starzyńska T. [Helicobacter pylori CagA(+) infection in gastric cancer patients]. *Pol Merkur Lekarski* 2002; **13**: 216-220

55 **Glupczynski Y**, Burette A, Goossens H, DePrez C, Butzler JP. Effect of antimicrobial therapy on the specific serological response to Helicobacter pylori infection. *Eur J Clin Microbiol Infect Dis* 1992; **11**: 583-588

56 **Shiao YH**, Rugge M, Correa P, Lehmann HP, Scheer WD. p53 alteration in gastric precancerous lesions. *Am J Pathol* 1994; **144**: 511-517

S- Editor Wang GP **L- Editor** Kumar M **E- Editor** Ma WH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5473-5478
World Journal of Gastroenterology ISSN 1007-9327

-765G > C *COX-2* polymorphism may be a susceptibility marker for gastric adenocarcinoma in patients with atrophy or intestinal metaplasia

Carina Pereira, Hugo Sousa, Paula Ferreira, Maria Fragoso, Luís Moreira-Dias, Carlos Lopes, Rui Medeiros, Mário Dinis-Ribeiro

Carina Pereira, Paula Ferreira, Molecular Oncology Group, Portuguese Institute of Oncology of Porto, Portugal
Maria Fragoso, Oncology Department, Portuguese Institute of Oncology of Porto, Portugal
Luís Moreira-Dias, Gastroenterology Department, Portuguese Institute of Oncology of Porto, Portugal
Hugo Sousa, Carlos Lopes, Rui Medeiros, Molecular Oncology Group, Portuguese Institute of Oncology of Porto, Portugal; and ICBAS, Abel Salazar Institute for the Biomedical Sciences, Porto, Portugal
Mário Dinis-Ribeiro, Gastroenterology Department, Portuguese Institute of Oncology of Porto, Portugal; and Cintesis / Faculty of Medicine University of Porto, Porto, Portugal
Supported by the Portuguese League Against Cancer (Liga Portuguesa Contra o Cancro-Núcleo Regional do Norte) and AstraZeneca Foundation
Correspondence to: Mário Dinis-Ribeiro, MD, PhD, Serviço de Gastrenterologia, Instituto Português de Oncologia do Porto FG EPE, Rua Dr. António Bernardino Almeida, 4200-072 Porto, Portugal. mario@med.up.pt
Telephone: +351-22-5084000-348 Fax: +351-22-5084001
Received: 2006-05-12 Accepted: 2006-06-16

Abstract

AIM: To investigate the relationship between the -765G > C *COX-2* polymorphism and the development of different gastric lesions: atrophy or intestinal metaplasia and gastric adenocarcinoma.

METHODS: A cross-sectional study was performed involving 320 Portuguese individuals (210 without evidence of neoplastic disease, 73 patients with gastric adenocarcinomas and 37 with atrophy or intestinal metaplasia) using a PCR-RFLP method.

RESULTS: -765C allele was overrepresented in the patients with gastric adenocarcinoma (51%) when compared either with the control group (38%) or patients with atrophy or intestinal metaplasia (27%). Callele was found to be very common in our population (0.22), and a multivariate logistic regression analysis revealed nearly 3-fold increased risk for the progression to gastric adenocarcinoma in patients with atrophy or intestinal metaplasia carrying the -765C allele (OR = 2.67, 95% CI = 1.03-6.93; P = 0.04).

CONCLUSION: -765C carrier status should be considered as another susceptibility marker for gastric adenocarcinoma development in patients with atrophy or intestinal metaplasia.

Key words: Gastric adenocarcinoma; Atrophy; Intestinal metaplasia; COX-2; Polymorphism; PCR-RFLP; Pharmacogenomic

Pereira C, Sousa H, Ferreira P, Fragoso M, Moreira-Dias L, Lopes C, Medeiros R, Dinis-Ribeiro M. -765G > C *COX-2* polymorphism may be a susceptibility marker for gastric adenocarcinoma in patients with atrophy or intestinal metaplasia. *World J Gastroenterol* 2006; 12(34): 5473-5478

http://www.wjgnet.com/1007-9327/12/5473.asp

INTRODUCTION

Gastric adenocarcinoma mortality rates have been decreasing in Europe[1], although, in Portugal it still remains one of the leading causes of cancer-related deaths (third in men and fourth in women). Portugal has one of the highest mortality rates of Europe and the highest in southern Europe, with values of 33.2 and 20.8 per 100 000/year in men and women, respectively[2].

Cyclooxygenase (COX), also known as prostaglandin endoperoxide synthase, is a rate-limiting enzyme that converts free arachidonic acid into important prostanoids (PGs) and eicosanoids such as prostaglandin H2[3]. There are at least two isoforms of COX identified[4]: *COX-1* is expressed constitutively in most cell types and is thought to be responsible for the maintenance of vascular homeostasis and gastroprotection[3,4]; and, *COX-2*, the inducible isoform of the enzyme, undetectable in most cells is readily induced by bacterial lipopolysaccharide (LPS), cytokine, growth factors, mitogens and tumor promoters[3-6].

Enhanced expression of *COX-2* has been observed in several forms of cancer[7-14], including gastric cancer and precancerous tissues[15-18]. *COX-2* over-expression plays an important role in the inhibition of apoptosis, tumor growth, angiogenesis, invasion and metastasis, which are considered to be important steps in cancer development[3,15,16,19-24].

Single Nucleotide Polymorphisms (SNP) are the most common form of genetic variants of the human genome[25], some of which might have functional effects on the susceptibility to the development of human cancers[26-33] by modifying the transcriptional activation.

Several polymorphisms in *COX-2* have been identified so far. However, only a few seemed to have a functional effect on the transcription. Recently, Papafili *et al*[34] described a new polymorphism in the promoter region of *COX-2*, characterized by a guanine (G) to cytosine (C) transition at position -765 (-765G > C). This polymorphism appears to disrupt a *Stimulatory protein 1* (Sp1) binding site, which is considered to be a positive activator of transcription and leads to a 30% reduction of the *COX-2* promoter activity *in vitro*[34]. With this evidence, only few investigations have been done involving *COX-2* polymorphisms either in cancer related studies[25,35-42] or other diseases[34,43-45].

The aim of this study was to determine the allelic frequencies of the -765G > C *COX-2* polymorphism in a northern Portuguese population and to investigate its association with the development of gastric lesions, such as, atrophic gastritis or intestinal metaplasia and gastric adenocarcinoma.

MATERIALS AND METHODS

Subjects

The -765G > C *COX-2* polymorphism was evaluated in a cross-sectional study performed in healthy individuals without clinical evidence of cancer (n = 210) and patients with known gastric lesions (n = 110), both from the northern region of Portugal attended at the Portuguese Institute of Oncology (Porto, Portugal).

The control group was formed by 75 females and 135 males (64%) with a median age of 51 years old. Patients were divided according to the type of lesion presented upon histopathological diagnosis after endoscopic multiple biopsies. Seventy three patients displayed lesions as severe as high-grade non-invasive neoplasia and intestinal type invasive gastric adenocarcinoma and 37 with lesions, such as atrophy or intestinal metaplasia that belong to a standardized follow-up since 2001[46]. The three different groups are characterized in Table 1. The group of patients with gastric adenocarcinoma included 27 females and 31 males (53%) with a mean age of 54 years and, and the group of patients with atrophy or intestinal metaplasia included 21 females and 13 males (38%) with a mean age of 61 years.

All samples were obtained with the permissions of the individuals before their inclusion in the study after informed consent according to the Declaration of Helsinki.

Sample DNA extraction

Blood samples were collected with a standard venipuncture technique using EDTA containing tubes. Genomic DNA was extracted from peripheral blood leukocytes by a standard Salting-out protocol[47].

Genotyping of -765G>C COX-2 polymorphism

The analysis of the -765G > C polymorphism was performed by PCR-based restriction fragment length polymorphism (PCR-RFLP) as previously described[43]. The primers used in the amplification were: CX2A (forward): 5'-ATT CTG GCC ATC GCC GCT TC-3' and CX2B (reverse) 5'-CTC CTT GTT TCT TGG AAA GAG ACG-3' (Metabion, Martinsried, Deutschland). The amplification conditions were 95℃ during 10 min for the initial denaturation step, followed by 35 cycles of denaturation at 94℃ (1 min), annealing at 59℃ (1 min) and extension at 72℃ (1 min). The final extension step consisted 10 min at 72℃. As a negative control PCR mix without DNA sample was used to ensure contamination free PCR product. Reaction products were digested with *Bsh*1236I restriction endonuclease (Fermentas, Vilnius, Lithuania) during 8 h at 37℃.

Results were observed in 3% agarose gel stained with ethidium bromide (Figure 1). Fragment sizes of 134 + 23 bp indicated a wild-type homozygous -756GG genotype, and an uncut fragment of 157 bp indicated the homozygous -765CC genotype. The presence of all the three bands (23, 134, and 157 bp) indicated a heterozygous -765GC genotype. The 23 bp fragment, resulting from the *Bsh*1236I restriction can not be distinguished from the primer-dimmer band in the agarose gel. Analysis of genotypes was independently performed by two of the authors (C.P. and P.F.). Cases with nonconcordant results between the two observers, or with the absence of a PCR product were rejected. Also a second PCR-RFLP analysis was performed in ten per cent of all samples to confirm the genotype.

Table 1 Characteristics of the participants: age, gender and type and stage of lesions

	Control n = 210	Gastric adenocarcinoma n = 73	Atrophy or intestinal metaplasia n = 37
Age (mean ± SD)	49.5 ± 18.0	54.2 ± 11.3	60.7 ± 10.9
Male Gender n (%)	135 (64)	36 (49)	15 (40)
Atrophy n (%)	Na	Na	3 (8)
Complete IM n (%)	Na	Na	4 (11)
Incomplete IM n (%)	Na	Na	30 (81)

Na: Not applicable; Atrophic chronic gastritis without intestinal metaplasia.

Variables

Individual's age and gender, type of gastric lesion (gastric adenocarcinoma or atrophy or intestinal metaplasia) or its absence, and *COX-2* alleles (G, C).

Statistical analysis

Data analysis was performed using the computer software *Statistical Package for Social Sciences-SPSS* for Windows (version 11.5). Chi-square analysis was used to compare categorical variables, using a 5% level of significance. Multivariate logistic regression analysis was used to estimate odds ratio (OR) and its 95% confidence interval (CI) as a measure of the association between Allele C carrier and the risk for the development of gastric lesion. Gender and age were included in multivariate analysis, and assessment for interaction was considered in the model.

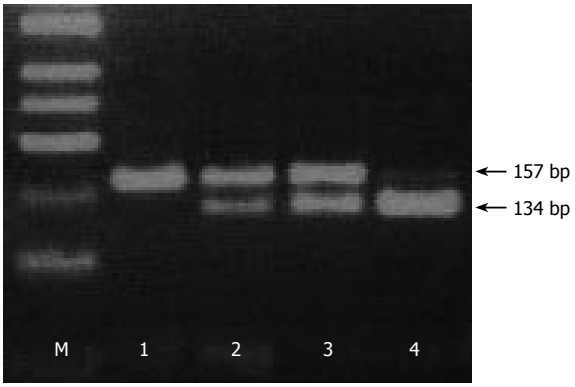


Figure 1 PCR-RFLP analysis of -765G > C *COX-2* polymorphism. M: 50 bp DNA ladder; 1: Homozygous -765CC genotype; 2, 3: Heterozygous -765GC genotype; 4: Homozygous -765GG genotype.

Table 3 Frequency distributions and Odds Ratio for risk of atrophy and intestinal metaplasia or gastric adenocarcinoma in -765 C carriers

Pathology	Genotype *n* (%)		OR (95% CI)		OR[2] (95% CI)
	GG	C carrier			
Controls	130 (62)	80 (38)	1.00 (Reference)	1.00 (Reference)[1]	
Atrophy or intestinal metaplasia	27 (73)	10 (27)	0.60 (0.28-1.31)	0.95 (0.91-0.99)[1]	1.00 (Reference)
Gastric adenocarcinoma	36 (49)	37 (51)	1.67 (0.98-2.86)	1.45 (0.84-2.64)[1]	2.67 (1.03-6.93)

[1] OR adjusted for age and gender in a multivariate logistic regression analysis; [2] OR adjusted for age and gender in a multivariate logistic regression analysis for the progression of atrophy or intestinal metaplasia into gastric cancer.

Table 2 Genotype distribution of *COX-2* -765G > C polymorphism

Genotype	Controls (*n* = 210)		Atrophy or intestinal metaplasia (*n* = 37)		Gastric adenocarcinoma (*n* = 73)
	n (%)	*P*[1]	*n* (%)	*P*[2]	*n* (%)
GG	130 (62)	0.197	27 (73)	0.018	36 (49)
GC	67 (32)	0.357	9 (24)	0.046	32 (44)
CC	13 (6)	0.398	1 (3)	0.339	5 (7)
C carrier	80 (38)	0.197	10 (27)	0.018	37 (51)

[1] *vs* Atrophy or intestinal metaplasia group (chi-square test); [2] *vs* Gastric adenocarcinoma group (chi-square test).

Table 4 C allele frequency and C carriers distribution in different countries

Country	*n*[1]	C allele frequency (%)	C carrier distribution (%)
America			
USA[39]	228	21	37
USA (African American)[39]	100	32	52
Europe			
Portugal (our study)	210	22	38
Italy[44]	864	28	50
UK[34]	454 (males)	14	25
Poland[43]	547	17	31
Australia			
Australia[45]	168	17	31
Asia			
Singapore[36]	1177	5	9
Japan[35]	241	2	5
China[18]	1270	2	4

[1] in control populations of the mentioned studies.

RESULTS

Allelic distribution of COX-2 polymorphism

The distribution of -765G > C *COX-2* genotypes is shown in Table 2. The frequency of the -765GG, GC and CC genotypes were 62%, 32% and 6%, respectively in controls, 49%, 44% and 7% in patients with gastric adenocarcinoma and 73%, 24% and 3% in patients with atrophic gastritis or intestinal metaplasia. All genotypic distributions are in Hardy-Weinberg equilibrium ($P > 0.05$). -765C carriers were more frequently found among those with gastric adenocarcinoma ($P = 0.04$) than the other groups.

Risk estimate for associated lesions and invasive gastric adenocarcinoma

Table 3 describes the Odds Ratio for the development of atrophic gastritis or intestinal metaplasia and gastric adenocarcinoma. We found no statistically significant risk for the development of either atrophy or intestinal metaplasia (OR = 0.60, 95% CI = 0.28-1.31; $P = 0.20$) or gastric adenocarcinoma (OR = 1.67, 95% CI = 0.98-2.86; $P = 0.06$). Although the results for the development of gastric lesions were not statistically significant, we observed a possible protective role for -765C carriers, and when the same analysis was adjusted for age and gender by a multivariate logistic regression analysis this protective effect disappeared (OR = 0.95, 95% CI = 0.91-0.99; $P = 0.01$). In contrast, we observed a nearly 3-fold increased risk for the progression of atrophy or intestinal metaplasia into gastric adenocarcinoma (OR = 2.67, 95% CI = 1.03-6.93; $P = 0.04$).

Furthermore, when we evaluated the distribution of gender in the two groups, atrophy or intestinal metaplasia and gastric adenocarcinoma, no statistically significant differences were observed ($P = 0.38$).

DISCUSSION

Enhanced expression of *COX-2* gene has been reported in several forms of cancer, including gastric precancerous and adenocarcinoma tissues[15-18]. This evidence suggests a role of *COX-2* in the carcinogenesis pathway, such as in the inhibition of apoptosis, tumour growth, angiogenesis, invasion and metastasis[3,15,16,19-24]. A -765G > C polymorphism on the promoter region of *COX-2* gene disrupts the Sp1 binding site[34] that may alter the susceptibility to develop cancer[36]. Our results revealed that C allele is extremely common (22%) in our population. Although only a few studies have been developed involving this *COX-2* polymorphism, the frequencies of the polymorphic variant seems to vary, especially among different ethnic populations (Table 4). These studies revealed that the C allele is

more frequent in Western countries, in Europe and America, than in Asian countries. Moreover, our results for the genotype frequencies are in concordance with other previously reported data in Caucasian populations.

-765G > C carriers and development of atrophic gastritis and intestinal metaplasia

In the present study, -765C carriers were slightly overrepresented in the control population (38%) when compared with patients with atrophy or intestinal metaplasia (27%). In fact, although not statistically significant, our results revealed a possible protective role for -765C carriers. Nevertheless, when adjusted for age and gender this protective role disappears (OR = 0.95, 95% CI = 0.91-0.99, P = 0.01) suggesting that this variant does not influence the development of gastric lesions such as atrophy or intestinal metaplasia. Although, the protective role is in agreement with previous studies, the small sample size may raise some statistical concerns to this observation. Papafili *et al*[34] showed that the promoter activity of the -765C allele is reduced to about 30% when compared to the -765G. In addition, Ulrich *et al*[38] revealed a marginal protection for the development of colorectal adenomatous and hyperplastic polyps when associated with the -765CC genotype. Thus, these results confirm the evidence that the depletion of the Sp1 binding site, considered a positive activator of *COX-2* transcription, caused by the -765G > C transition, modifies the transcriptional activation of *COX-2*[34].

-765G > C polymorphism and the development of gastric adenocarcinoma in patients with atrophic gastritis and intestinal metaplasia

Gastric cancer developed upon a multistep process from chronic active gastritis, gastric glandular atrophy (GA), intestinal metaplasia (IM), dysplasia and gastric cancer[46]. In a recent work, it was suggested that *COX-2* expression increases as it progresses from initial gastric lesions to gastric cancer, providing evidence that *COX-2* might contribute to an early event in gastric carcinogenesis[48]. Another approach attempt to understand the influence of the *COX-2* -765G > C polymorphism has in the progression from atrophy or intestinal metaplasia lesions to gastric adenocarcinoma. We observed a nearly 3-fold increased risk of progression from gastric lesions into gastric cancer (OR = 2.67, 95% CI = 1.03-6.93; P = 0.04). This result is consistent with previously cancer-related studies that also revealed that -765C allele carriers had increased risk for the development of those diseases[36,49]. More recently, Zhang *et al*[42] described a 2-fold increased risk for the development of esophageal squamous cell carcinoma due to increased expression of *COX-2* mRNA in -765G > C heterozygous. Although the exact molecular mechanism by which *COX-2* polymorphism may affect the risk of gastric adenocarcinoma development is still unclear, studies in the *COX-2* promoter revealed that *COX-2* transcription is activated by E2 promoter binding factor 1 (E2F1)[50], which is dependent on the transactivation and DNA-binding domains of E2F1[51]. So the ability of this polymorphism to create an E2F binding site, essential for the expression of several genes[43], might help us to understand why we observed increased risk.

In conclusion, all these findings suggest that different physiological/pathological conditions, as well as cell type, could determine the influence that -765G > C *COX-2* polymorphism has in the development of human diseases, by the modification of the binding sites for the transcription factors[18]. The contribution of genetic polymorphism to the risk of gastric adenocarcinoma may be dependent on the population in study, as well on several environmental and dietary factors that influence that population. So, we hypothesize that each population has to evaluate its own genetic profile for cancer risk that may help to understand the geographic and racial differences reported for gastric adenocarcinoma[52]. Furthermore, *COX-2* polymorphisms may be involved in different individual drug response[53,54] and may be explored as for in clinical trials to select those individual to be submitted to *COX-2* inhibition. Moreover, the definition of a pharmacogenomic profile using molecular studies may help to the development of a personalized treatment or quimioprevention.

To the best of our knowledge, this is the first report that evaluates the -765G > C *COX-2* polymorphisms and gastric adenocarcinoma development worldwide, which also considers the progression from gastric lesions, such as atrophy or intestinal metaplasia, to gastric adenocarcinoma. We theorise that once the lesions are installed, -765C carriers are at risk of progression into gastric adenocarcinoma. However, our results should be cautiously interpreted as they report a cross-sectional design. Therefore, we suggest that -765G > C polymorphism should be used in a large cohort study among patients with atrophy or intestinal metaplasia as a susceptibility marker for gastric adenocarcinoma and to confirm the real meaning of this genetic alteration in gastric adenocarcinoma development.

REFERENCES

1 **Pinheiro PS**, Tyczyński JE, Bray F, Amado J, Matos E, Parkin DM. Cancer incidence and mortality in Portugal. *Eur J Cancer* 2003; **39**: 2507-2520

2 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108

3 **Wang D**, Mann JR, DuBois RN. The role of prostaglandins and other eicosanoids in the gastrointestinal tract. *Gastroenterology* 2005; **128**: 1445-1461

4 **Markenson J**. Clinical Implications of Cyclooxygenase Enzymes: COX-1/COX-2 Role of the New NSAIDs. *Cancer Control* 1999; **6**: 22-25

5 **McAdam BF**, Mardini IA, Habib A, Burke A, Lawson JA, Kapoor S, FitzGerald GA. Effect of regulated expression of human cyclooxygenase isoforms on eicosanoid and isoeicosanoid production in inflammation. *J Clin Invest* 2000; **105**: 1473-1482

6 **Bakhle YS**. COX-2 and cancer: a new approach to an old problem. *Br J Pharmacol* 2001; **134**: 1137-1150

7 **Chan G**, Boyle JO, Yang EK, Zhang F, Sacks PG, Shah JP, Edelstein D, Soslow RA, Koki AT, Woerner BM, Masferrer JL, Dannenberg AJ. Cyclooxygenase-2 expression is up-regulated in squamous cell carcinoma of the head and neck. *Cancer Res* 1999; **59**: 991-994

8 **Gupta S**, Srivastava M, Ahmad N, Bostwick DG, Mukhtar H. Over-expression of cyclooxygenase-2 in human prostate adenocarcinoma. *Prostate* 2000; **42**: 73-78

9 **Boström PJ,** Aaltonen V, Söderström KO, Uotila P, Laato M.

Expression of cyclooxygenase-1 and -2 in urinary bladder carcinomas *in vivo* and *in vitro* and prostaglandin E2 synthesis in cultured bladder cancer cells. *Pathology* 2001; **33**: 469-474
10 **Kokawa A**, Kondo H, Gotoda T, Ono H, Saito D, Nakadaira S, Kosuge T, Yoshida S. Increased expression of cyclooxygenase-2 in human pancreatic neoplasms and potential for chemoprevention by cyclooxygenase inhibitors. *Cancer* 2001; **91**: 333-338
11 **Ferrandina G**, Legge F, Ranelletti FO, Zannoni GF, Maggiano N, Evangelisti A, Mancuso S, Scambia G, Lauriola L. Cyclooxygenase-2 expression in endometrial carcinoma: correlation with clinicopathologic parameters and clinical outcome. *Cancer* 2002; **95**: 801-807
12 **Niki T**, Kohno T, Iba S, Moriya Y, Takahashi Y, Saito M, Maeshima A, Yamada T, Matsuno Y, Fukayama M, Yokota J, Hirohashi S. Frequent co-localization of Cox-2 and laminin-5 gamma2 chain at the invasive front of early-stage lung adenocarcinomas. *Am J Pathol* 2002; **160**: 1129-1141
13 **Garcea G**, Sharma RA, Dennison A, Steward WP, Gescher A, Berry DP. Molecular biomarkers of colorectal carcinogenesis and their role in surveillance and early intervention. *Eur J Cancer* 2003; **39**: 1041-1052
14 **Brown JR**, DuBois RN. Cyclooxygenase as a target in lung cancer. *Clin Cancer Res* 2004; **10**: 4266s-4269s
15 **Li HX**, Chang XM, Song ZJ, He SX. Correlation between expression of cyclooxygenase-2 and angiogenesis in human gastric adenocarcinoma. *World J Gastroenterol* 2003; **9**: 674-677
16 **Chen XL**, Su BS, Sun RQ, Zhang J, Wang YL. Relationship between expression and distribution of cyclooxygenase-2 and bcl-2 in human gastric adenocarcinoma. *World J Gastroenterol* 2005; **11**: 1228-1231
17 **Yu JR**, Wu YJ, Qin Q, Lu KZ, Yan S, Liu XS, Zheng SS. Expression of cyclooxygenase-2 in gastric cancer and its relation to liver metastasis and long-term prognosis. *World J Gastroenterol* 2005; **11**: 4908-4911
18 **Zhang JT**, Wang MW, Zhu ZL, Huo XH, Chu JK, Cui DS, Qiao L, Yu J. Increased expression of cyclooxygenase-2 in first-degree relatives of gastric cancer patients. *World J Gastroenterol* 2005; **11**: 4918-4922
19 **Leahy KM**, Koki AT, Masferrer JL. Role of cyclooxygenases in angiogenesis. *Curr Med Chem* 2000; **7**: 1163-1170
20 **Uefuji K**, Ichikura T, Mochizuki H. Cyclooxygenase-2 expression is related to prostaglandin biosynthesis and angiogenesis in human gastric cancer. *Clin Cancer Res* 2000; **6**: 135-138
21 **Dempke W**, Rie C, Grothey A, Schmoll HJ. Cyclooxygenase-2: a novel target for cancer chemotherapy? *J Cancer Res Clin Oncol* 2001; **127**: 411-417
22 **Howe LR**, Subbaramaiah K, Brown AM, Dannenberg AJ. Cyclooxygenase-2: a target for the prevention and treatment of breast cancer. *Endocr Relat Cancer* 2001; **8**: 97-114
23 **Cao Y**, Prescott SM. Many actions of cyclooxygenase-2 in cellular dynamics and in cancer. *J Cell Physiol* 2002; **190**: 279-286
24 **Wang D**, DuBois RN. Cyclooxygenase 2-derived prostaglandin E2 regulates the angiogenic switch. *Proc Natl Acad Sci USA* 2004; **101**: 415-416
25 **Hu Z**, Miao X, Ma H, Wang X, Tan W, Wei Q, Lin D, Shen H. A common polymorphism in the 3'UTR of cyclooxygenase 2/prostaglandin synthase 2 gene and risk of lung cancer in a Chinese population. *Lung Cancer* 2005; **48**: 11-17
26 **Medeiros R**, Morais A, Vasconcelos A, Costa S, Pinto D, Oliveira J, Lopes C. The role of vitamin D receptor gene polymorphisms in the susceptibility to prostate cancer of a southern European population. *J Hum Genet* 2002; **47**: 413-418
27 **Ferreira PM**, Medeiros R, Vasconcelos A, Costa S, Pinto D, Morais A, Oliveira J, Lopes C. Association between CYP2E1 polymorphisms and susceptibility to prostate cancer. *Eur J Cancer Prev* 2003; **12**: 205-211
28 **Ribeiro R**, Vasconcelos A, Costa S, Pinto D, Morais A, Oliveira J, Lobo F, Lopes C, Medeiros R. Overexpressing leptin genetic polymorphism (-2548 G/A) is associated with susceptibility to prostate cancer and risk of advanced disease. *Prostate* 2004; **59**: 268-274
29 **Pinto D**, Vasconcelos A, Costa S, Pereira D, Rodrigues H, Lopes C, Medeiros R. HER2 polymorphism and breast cancer risk in Portugal. *Eur J Cancer Prev* 2004; **13**: 177-181
30 **Freitas-Silva M**, Pereira D, Coelho C, Bicho M, Lopes C, Medeiros R. Angiotensin I-converting enzyme gene insertion/deletion polymorphism and endometrial human cancer in normotensive and hypertensive women. *Cancer Genet Cytogenet* 2004; **155**: 42-46
31 **Coelho A**, Matos A, Catarino R, Pinto D, Pereira D, Lopes C, Medeiros R. Protective role of the polymorphism CCR2-64I in the progression from squamous intraepithelial lesions to invasive cervical carcinoma. *Gynecol Oncol* 2005; **96**: 760-764
32 **Catarino R**, Matos A, Pinto D, Pereira D, Craveiro R, Vasconcelos A, Lopes C, Medeiros R. Increased risk of cervical cancer associated with cyclin D1 gene A870G polymorphism. *Cancer Genet Cytogenet* 2005; **160**: 49-54
33 **Duarte I**, Santos A, Sousa H, Catarino R, Pinto D, Matos A, Pereira D, Moutinho J, Canedo P, Machado JC, Medeiros R. G-308A TNF-alpha polymorphism is associated with an increased risk of invasive cervical cancer. *Biochem Biophys Res Commun* 2005; **334**: 588-592
34 **Papafili A**, Hill MR, Brull DJ, McAnulty RJ, Marshall RP, Humphries SE, Laurent GJ. Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. *Arterioscler Thromb Vasc Biol* 2002; **22**: 1631-1636
35 **Hamajima N**, Takezaki T, Matsuo K, Saito T, Inoue M, Hirai T, Kato T, Ozeki J, Tajima K. Genotype Frequencies of Cyclooxygenease 2 (COX2) Rare Polymorphisms for Japanese with and without Colorectal Cancer. *Asian Pac J Cancer Prev* 2001; **2**: 57-62
36 **Koh WP**, Yuan JM, van den Berg D, Lee HP, Yu MC. Interaction between cyclooxygenase-2 gene polymorphism and dietary n-6 polyunsaturated fatty acids on colon cancer risk: the Singapore Chinese Health Study. *Br J Cancer* 2004; **90**: 1760-1764
37 **Goodman JE**, Bowman ED, Chanock SJ, Alberg AJ, Harris CC. Arachidonate lipoxygenase (ALOX) and cyclooxygenase (COX) polymorphisms and colon cancer risk. *Carcinogenesis* 2004; **25**: 2467-2472
38 **Ulrich CM**, Whitton J, Yu JH, Sibert J, Sparks R, Potter JD, Bigler J. PTGS2 (COX-2) -765G > C promoter variant reduces risk of colorectal adenoma among nonusers of nonsteroidal anti-inflammatory drugs. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 616-619
39 **Brosens LA**, Iacobuzio-Donahue CA, Keller JJ, Hustinx SR, Carvalho R, Morsink FH, Hylind LM, Offerhaus GJ, Giardiello FM, Goggins M. Increased cyclooxygenase-2 expression in duodenal compared with colonic tissues in familial adenomatous polyposis and relationship to the -765G -> C COX-2 polymorphism. *Clin Cancer Res* 2005; **11**: 4090-4096
40 **Kang S**, Kim YB, Kim MH, Yoon KS, Kim JW, Park NH, Song YS, Kang D, Yoo KY, Kang SB, Lee HP. Polymorphism in the nuclear factor kappa-B binding promoter region of cyclooxygenase-2 is associated with an increased risk of bladder cancer. *Cancer Lett* 2005; **217**: 11-16
41 **Siezen CL**, van Leeuwen AI, Kram NR, Luken ME, van Kranen HJ, Kampman E. Colorectal adenoma risk is modified by the interplay between polymorphisms in arachidonic acid pathway genes and fish consumption. *Carcinogenesis* 2005; **26**: 449-457
42 **Zhang X**, Miao X, Tan W, Ning B, Liu Z, Hong Y, Song W, Guo Y, Zhang X, Shen Y, Qiang B, Kadlubar FF, Lin D. Identification of functional genetic variants in cyclooxygenase-2 and their association with risk of esophageal cancer. *Gastroenterology* 2005; **129**: 565-576
43 **Szczeklik W**, Sanak M, Szczeklik A. Functional effects and gender association of COX-2 gene polymorphism G-765C in bronchial asthma. *J Allergy Clin Immunol* 2004; **114**: 248-253
44 **Cipollone F**, Toniato E, Martinotti S, Fazia M, Iezzi A, Cuccurullo C, Pini B, Ursi S, Vitullo G, Averna M, Arca M, Montali A, Campagna F, Ucchino S, Spigonardo F, Taddei S, Virdis A, Ciabattoni G, Notarbartolo A, Cuccurullo F, Mezzetti A. A polymorphism in the cyclooxygenase 2 gene as an inherited

protective factor against myocardial infarction and stroke. *JAMA* 2004; **291**: 2221-2228

45 **Shi J**, Misso NL, Duffy DL, Thompson PJ, Kedda MA. A functional polymorphism in the promoter region of the cyclooxygenase-2 gene is not associated with asthma and atopy in an Australian population. *Clin Exp Allergy* 2004; **34**: 1714-1718

46 **Dinis-Ribeiro M**, Lopes C, da Costa-Pereira A, Guilherme M, Barbosa J, Lomba-Viana H, Silva R, Moreira-Dias L. A follow up model for patients with atrophic chronic gastritis and intestinal metaplasia. *J Clin Pathol* 2004; **57**: 177-182

47 **Müllenbach R**, Lagoda PJ, Welter C. An efficient salt-chloroform extraction of DNA from blood and tissues. *Trends Genet* 1989; **5**: 391

48 **Sun WH**, Yu Q, Shen H, Ou XL, Cao DZ, Yu T, Qian C, Zhu F, Sun YL, Fu XL, Su H. Roles of Helicobacter pylori infection and cyclooxygenase-2 expression in gastric carcinogenesis. *World J Gastroenterol* 2004; **10**: 2809-2813

49 **Panguluri RC**, Long LO, Chen W, Wang S, Coulibaly A, Ukoli F, Jackson A, Weinrich S, Ahaghotu C, Isaacs W, Kittles RA. COX-2 gene promoter haplotypes and prostate cancer risk. *Carcinogenesis* 2004; **25**: 961-966

50 **Kovesdi I**, Reichel R, Nevins JR. Role of an adenovirus E2 promoter binding factor in E1A-mediated coordinate gene control. *Proc Natl Acad Sci USA* 1987; **84**: 2180-2184

51 **Davis JN**, McCabe MT, Hayward SW, Park JM, Day ML. Disruption of Rb/E2F pathway results in increased cyclooxygenase-2 expression and activity in prostate epithelial cells. *Cancer Res* 2005; **65**: 3633-3642

52 **Crew KD**, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol* 2006; **12**: 354-362

53 **Cambria-Kiely JA**, Gandhi PJ. Aspirin resistance and genetic polymorphisms. *J Thromb Thrombolysis* 2002; **14**: 51-58

54 **Gonzalez-Conejero R**, Rivera J, Corral J, Acuña C, Guerrero JA, Vicente V. Biological assessment of aspirin efficacy on healthy individuals: heterogeneous response or aspirin failure? *Stroke* 2005; **36**: 276-280

S- Editor Pan BR **L- Editor** Karam SM **E- Editor** Bi L

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5479-5482
World Journal of Gastroenterology ISSN 1007-9327

H pylori infection among 1000 southern Iranian dyspeptic patients

Mahmood Reza Hashemi, Mohammad Rahnavardi, Bavand Bikdeli, Mohsen Dehghani Zahedani

Mahmood Reza Hashemi, Department of Gastroenterology, Hormozgan University of Medical Sciences, Bandarabbas, Artesh University of Medical Sciences, Tehran, Iran
Mohammad Rahnavardi, Bavand Bikdeli, Educational Development Center, Artesh University of Medical Sciences, Tehran, Iran
Mohsen Dehghani Zahedani, Department of Pathology, Bandarabbas University of Medical Sciences, Bandarabbas, Iran
Correspondence to: Mohammad Rahnavardi, MD, 33, unit 16, Golshahr Blvd, Africa Blvd, Tehran 1915665144, Iran. rahnavardi@gmail.com
Telephone: +98-912-3092024 Fax: +98-21-88797363
Received: 2006-06-13 Accepted: 2006-07-07

Abstract

AIM: To describe the frequency of *H pylori* infection among 1000 southern Iranian dyspeptic patients.

METHODS: A prospective study was performed in a referral hospital in south of Iran from 1999 to 2005. One thousand dyspeptic patients (518 males, mean ± SD age of 49.12 ± 12.82 years) consecutively underwent upper gastrointestinal endoscopy. Multiple gastric antral biopsy samples were taken from all patients for rapid urease test and histopathologic examination (96.9% satisfactory samples). Patients were considered *H pylori*-infected if one or both tests were positive.

RESULTS: Six hundred and seventy-one patients (67.1%, 95% confidence interval [CI]: 64.2%-70.0%) were *H pylori*-infected. *H pylori* positivity was significantly more frequent in patients with peptic ulcer disease (PUD) than in those with non-ulcer dyspepsia ($P < 0.001$). Male-to-female ratio for duodenal and gastric ulcers was 2.7:1 and 1.5:1, respectively. Moreover, the duodenal-to-gastric ulcer ratio was 1.95:1. The frequency of *H pylori* infection among those with endoscopic diagnosis of gastritis, duodenal ulcer, gastric ulcer, and normal mucosa was 70.1% (398/568), 86.2% (150/174), 71.9% (64/89), and 33.5% (54/161), respectively. *H pylori* infection, male sex, and older age were independently associated with PUD in multivariate analysis. *H pylori* positivity was associated with chronic gastritis, and chronic active gastritis with odds ratios of 34.21 (95% CI: 12.19%-96.03%) and 81.21 (95% CI: 28.85%-228.55%), respectively.

CONCLUSION: *H pylori* and PUD are highly frequent in dyspeptic patients from south of Iran. *H pylori* is a cardinal risk factor for chronic active or inactive gastritis.

Key words: Dyspepsia; *H pylori*; Iran

Hashemi MR, Rahnavardi M, Bikdeli B, Dehghani Zahedani M. *H pylori* infection among 1000 southern Iranian dyspeptic patients. *World J Gastroenterol* 2006; 12(34): 5479-5482

http://www.wjgnet.com/1007-9327/12/5479.asp

INTRODUCTION

H pylori is a major cause of gastritis and peptic ulcer disease (PUD), and has been implicated in the development of gastric malignancy[1-3]. The prevalence of *H pylori*, a worldwide infection, varies greatly among countries and among population groups within the same country[4]. *H pylori* is highly prevalent in the developing countries[5-11] and is common in 57%-91% of Iranian population[5,12-14]. However, studies regarding *H pylori* prevalence in different regions of Iran, a country with a miscellaneous climate, are limited[5,12-16]. Thus, we studied the frequency of *H pylori* infection among 1000 southern Iranian dyspeptic patients. Furthermore, we report *H pylori* association with different histopathologic and upper gastrointestinal endoscopy (UGIE) findings in this large number of patients.

MATERIALS AND METHODS

Subjects

A prospective study was performed in the Gastroenterology Division, Shahid-Mohammadi Referral Hospital, Bandarabbas, Iran from October 1999 to August 2005. One thousand consecutive patients (518 males, mean ± SD age of 49.12 ± 12.82 years, range 13-89 years) with dyspepsia underwent UGIE. Those with age over 12 years, no prior gastric surgery, no active bleeding, and no consumption of antibiotics, bismuth preparations, or proton pump inhibitors in the 4 wk prior to UGIE were eligible to enter the study. All patients enrolled gave their informed consent and the study was performed according to the Declaration of Helsinki.

Methods

UGIE reported signs of gastritis, PUD, polyp, mucosal atrophy, and tumor. When PUD was present, concomitant gastritis was not mentioned in the study questionnaire. Moreover, when none of the above-mentioned pathologic

Table 1 Numbers and proportions of *H pylori* infected patients

	n	%	95% CI
Total	671/1000	67.1	64.2-70.0
Age (yr)[a]			
⩽ 20	57/105	54.3	44.7-63.9
21-50	380/559	68.0	64.1-71.9
⩾ 51	234/336	69.6	64.7-74.5
Sex			
Male	362/518	69.9	66.0-73.8
Female	309/482	64.1	59.8-68.4
UGIE finding[b]			
PUD	214/263	81.4	76.7-86.1
NUD	453/732	61.9	58.4-65.4
Tumor	4/5	80.0	40.8-100

PUD: Peptic ulcer disease; NUD: Nonulcer dyspepsia. [a]$P < 0.05$ between groups regarding *H pylor*i infection; [b]$P < 0.001$ between PUD and NUD groups regarding *H pylori* infection.

features was present, the UGIE examination was considered normal. During UGIE, at least three biopsy specimens were taken from the antrum lesser curvature mucosa 3-4 cm proximal to the pylorus. One specimen was for rapid urease test (RUT). The RUT was monitored for color change up to 6 h after addition of the gastric tissue. The test was scored as positive if the color changed from yellow to red. The remaining two specimens were fixed in formaldehyde and submitted for histologic examination and HE staining. Nine hundred and sixty-nine antral mucosa biopsies (96.9%) were satisfactory for histopathological examination. One experienced pathologist, blinded to the results of RUT and UGIE, evaluated the coded samples. Thirty percent of coded samples ($n = 291$) were randomly evaluated by a second experienced pathologist. When the results were different ($n = 11$), the slides were discussed in joint sessions where a third pathologist was also present. The slides were observed under an optical microscope at several magnifications (including oil immersion). An increase in lymphocytes and plasma cells in the lamina propria characterized the gastritis as chronic. Activity in the context of chronic gastritis referred to the density of neutrophil polymorphs in the lamina propria, gastric pits, and surface epithelium. The presence of any pathology in at least one biopsy sample was considered a positive finding. The absence of abnormal findings in all specimens was regarded as normal. Detection of any *H pylori* was contemplated evidence of infection. Patients were considered *H pylori* positive if RUT and/or histology were/was positive. When the histologic sample was unsatisfactory, patient's of *H pylori* infection was determined by the RUT.

The frequency of *H pylori* was evaluated by means of cross tables regarding different age, sex, endoscopic and histopathologic diagnoses and tested by chi-square (χ^2) test and Fisher's exact test. Multivariate analysis was performed by entering sex, age groups, and *H pylori* status in a logistic regression model to identify the independent risk factors for endoscopic diagnoses of duodenal ulcer, gastric ulcer, and gastritis (normal endoscopy was set as a reference category). The same model was built to identify the independent risk factors for histopathologic diagnoses (normal mucosa was set as a reference category). For all point prevalences, 95% confidence intervals (CI) were calculated. Statistical significance was set at $P < 0.05$.

Table 2 Histological findings of satisfactory 969 antral biopsies

Histological finding	*n*	% (95% CI)
Normal mucosa	84	8.7 (6.9-10.4)
Chronic inactive gastritis	365	37.7 (34.6-40.7)
Chronic active gastritis	456	47.1 (43.9-50.2)
Atrophic changes	242	25.0 (23.6-27.7)
Intestinal metaplasia/malignancy	81	8.9 (7.1-10.7)
Glandular dysplasia	1	0.1 (-0.1-0.3)
Adenocarcinoma	4	0.4 (0.0-0.8)

RESULTS

Six hundred and seventy-one patients (67.1%, Table 1) were *H pylori*-infected. PUD was found in 263 enrolled patients (26.3%, 95% CI: 23.6%-29.0%). Those with PUD were significantly more *H pylori*-infected than those with non-ulcer dyspepsia (NUD) ($P < 0.001$, Table 1). Fisher's exact test displayed that PUD was more frequent in male patients than in female patients (68.8%, 95% CI: 63.2%-74.5%) *vs* 31.2% (95% CI: 25.5%-36.8%) ($P < 0.001$). Male-to-female ratio for duodenal and gastric ulcers was 2.7:1 (127/47) and 1.5:1 (54/35), respectively. While the duodenal-to-gastric ulcer ratio was 1.9:1 (174/89), endoscopic signs of gastritis were evident in 568 (56.8%), polyp in 7 (0.7%), mucosal atrophy in 5 (0.5%), raised/thickened area in 5 (0.5%), and accompanying duodenal and gastric ulcers in 3 (0.3%) patients. Thus, 161 patients (16.1%) had normal endoscopic results. A histologic diagnosis of chronic active gastritis was assigned to 47.1% of biopsy samples. Table 2 summarizes the histopathological findings.

While *H pylori* was frequent in 33.5% of those with normal UGIE, 86.2% of those with duodenal ulcer were *H pylori*-infected ($P < 0.001$, Table 3). Those with histologic diagnosis of chronic active gastritis were significantly more *H pylori*-infected than those with chronic inactive gastritis ($P < 0.001$, Table 3). Table 3 summarizes the frequency of *H pylori* infection regarding different endoscopic and histopathologic findings. Multivariate logistic regression analysis proved *H pylori* positivity, older age, and male gender as predictors of duodenal ulcer, gastric ulcer, and gastritis (Table 4). The second model to identify the risk factors for histopathologic findings, had a 56.1% agreement between predicted and observed results and the amount of variance accounted for 21.4% (Cox & Snell) - 25.3% (Nagelkerke). This model suggested that *H pylori* positivity was associated with chronic gastritis, and chronic active gastritis with odds ratios (OR) of 34.21 (95% CI: 12.19%-96.03%) and 81.21 (95% CI: 28.85%-228.55%), respectively.

DISCUSSION

In the developing world, *H pylori* is a challenging health problem as 20% prevalence of *H pylori* infection among adolescents in the United States pales in comparison with infection rates exceeding 90% by five years of age in parts of the developing world[17]. One study in northwest of

Table 3 *H pylori* infection regarding endoscopic and histologic diagnoses *n* (%)

Diagnosis	*H pylori* infection		
	Negative	Positive	Total
Endoscopic findings			
Normal	107 (66.5)	54 (33.5)	161
Gastritis	170 (29.9)	398 (70.1)[b]	568
Duodenal Ulcer	24 (13.8)	150 (86.2)[b,d]	174
Gastric Ulcer	25 (28.1)	64 (71.9)[b]	89
Histopathologic findings			
Normal mucosa	80 (95.2)	4 (4.8)	84
Chronic inactive gastritis	130 (35.6)	235 (64.4)[f]	365
Chronic active gastritis	87 (19.1)	369 (80.9)[h]	456
Atrophic changes	76 (31.4)	166 (68.6)[f]	242
Metaplasia/dysplasia/adenocarcinoma	15 (17.4)	71 (82.6)[f]	86

[b]$P < 0.001$ *vs* normal endoscopic findings; [d]$P < 0.001$ *vs* gastritis and gastric ulcer; [f]$P < 0.001$ *vs* normal histopathologic findings; [h]$P < 0.001$ *vs* histopathologic findings and chronic inactive gastritis (Fisher's exact test).

Table 4 Multivariate logistic regression of selected model variables on endoscopic findings[1]

Variable	B	SE	Wald test	*P*	Odds ratio (95% CI)
Duodenal ulcer					
Sex (male)	1.91	0.26	55.14	< 0.001	6.75 (4.08-11.17)
Age (in three ascending groups)	0.59	0.19	9.211	< 0.01	1.80 (1.23-2.63)
H pylori (+)	2.54	0.28	79.21	< 0.001	12.66 (7.24-22.14)
Constant	-3.75	0.50			
Gastric Ulcer					
Sex (male)	1.34	0.28	22.13	< 0.001	3.83 (2.19-6.69)
Age (in three ascending groups)	0.65	0.22	8.49	< 0.01	1.91 (1.24-2.95)
H pylori (+)	1.63	0.29	30.74	< 0.001	5.12 (2.87-9.11)
Constant	-3.45	0.56			
Gastritis					
Sex (male)	0.91	0.20	20.15	< 0.001	2.48 (1.67-3.68)
Age (in three ascending groups)	0.65	0.15	18.35	< 0.001	1.92 (1.42-2.58)
H pylori (+)	1.54	0.19	61.81	< 0.001	4.65 (3.17-6.82)
Constant	-1.30	0.361			

[1]Normal endoscopy was set as a reference category; agreement between predicted and observed results: 57.2%; the amount of variance accounted for 17.6% (Cox & Snell)- 19.4% (Nagelkerke).

Iran, a region with the highest mortality rate from gastric cancer throughout the country, reported that *H pylori* infection occurs in 89.2% (883/990) of the residents[5]. Other surveys in different age groups from various regions of the country reported that *H pylori* infection occurs in 57%-91% of the study subjects[12-14]. In this prospective survey, we report *H pylori* infection in 67.1% of 1000 enrolled dyspeptic patients from south of Iran. Variation in study powers as well as ethnicity, place of birth, socioeconomic factors, diet, occupation, smoking, or alcohol consumption habits among study populations may be the reasons for erratic rates of *H pylori* infection reported from the country[6,18]. Similarly, *H pylori* seems to be a health problem in the neighboring regions of Iran. In India, *H pylori* is positive in 38 (56.7%) asymptomatic individuals and in 49 (61.3%) symptomatic individuals[6]. In Saudi-Arabia, *H pylori* is present in 54.9% of gastric biopsies from 488 dyspeptic patients[7]. In Yemen, 82.2% of 275 dyspeptic patients are *H pylori*-infected[8]. In Jordan, *H pylori* is frequent in 82% of 197 study subjects[9]. In United Arab Emirates[10] and in Kuwait[11], 90.39% of 437 and 96.6% of 204 studied subjects are infected with *H pylori*, respectively.

About a quarter of dyspeptic patients in this study were proved to have PUD. Nevertheless, in another large cohort of residents in northwest of Iran, the frequency of PUD is just 4.9%[5]. This relatively low frequency of PUD might be due to the enrollment of unnecessarily dyspeptic subjects in the latter survey. PUD frequencies are divergent in reports from different countries. In a literature review by the American Gastroenterology Association[19], 19 out of 41 studies report duodenal ulcer in $\geqslant$ 10% of dyspeptic patients and the overall prevalence of PUD in these groups of symptomatic patients is $\geqslant$ 15% in 21 studies. Duodenal ulcer was approximately twice as common as gastric ulcer in the present survey, which is in quite contrast to the 12:1 ratio reported from India[6]. Moreover, *H pylori* infection is significantly more frequent in PUD than in NUD patients. Regarding the significantly higher rate of *H pylori* infection in those with duodenal (86.2%) and gastric (71.9%) ulcers in comparison with the subjects with normal endoscopic findings (33.5%) (Table 3), also the significant association of *H pylori* positivity with duodenal (OR: 12.66) as well as gastric ulcers (OR: 5.12) (Table 4), *H pylori* can be introduced as an aetiological agent for PUD, thus strengthening prior findings[10,20,21]. Furthermore, current evidence shows the cardinal role of *H pylori* in the pathogenesis of PUD[22-24]. In favor of the results of some studies[25-27] and against the findings of others[6,28,29], we found significantly more *H pylori* infections in male than in female PUD subjects, but *H pylori* was not significantly more prevalent in males. Regarding these two latter findings, one may deduce that *H pylori* infection independently results in PUD in males more frequent than in females. The regression model also confirms this judgment as entering both sex and *H pylori* status in the model showed an independent significant role of both factors in prediction of different endoscopic findings (Table 4). Nevertheless, despite more than a 50% agreement between the predicted and observed results of both models in this survey, outcomes should be carefully interpreted due to the limited factors entered into these models as other factors with a possible predictive role were beyond the scope of the current study and thus were not entered into the model.

In the present study, histologic findings of chronic active and inactive gastritis were frequent in about 85% of dyspeptic subjects, which is comparable with the previous 77.8% of chronic active gastritis in northwest of Iran[5], 80.6% and 67% of chronic gastritis in Saudi-Arabia[30] and India[6], respectively. Similarly, *H pylori* with a significantly higher frequency in those with chronic active and inactive gastritis compared to those with normal histology, showed a strong association with chronic active gastritis (OR: 81.21) and chronic inactive (OR: 34.21) gastritis, although more frequent in those with chronic active gastritis, which is suggestive of its causative role in chronic gastritis and gastritis activity. Despite some doubts[31], *H pylori* is globally believed to have a fundamental role in the pathogenesis of gastric cancer[3,32,33]. Chronic *H pylori* gastritis leads in more

than half of the affected subjects to a gradual loss of the glandular structures with its specialized cells and a collapse of the reticulin skeleton of the mucosa, a condition of atrophic gastritis[34]. Indeed, the most common type of gastric cancer, the intestinal type, is preceded by chronic atrophic gastritis, which is 22%-37% prevalent in asymptomatic European adult subjects[35]. In this survey, a quarter of satisfactory antral biopsies were proved to have atrophic changes in histology, about two thirds of which were associated with *H pylori* infection. Compared to our findings in south of Iran as well as those in the developed world[35], northwest of Iran with a relatively higher frequency of atrophic changes in the antral biopsies of the sampled population (45.2%)[5] might be at a higher risk of prevalence of gastric malignancies in the near future, an alarming condition that necessitates further investigations and thoughtful interventions.

In conclusion, *H pylori* and PUD are frequent in dyspeptic patients from south of Iran. *H pylori* infection, male sex, and older age are independently associated with PUD. *H pylori* is associated with chronic gastritis and even more with chronic active gastritis.

REFERENCES

1 Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983; **1**: 1273-1275
2 **Forman D**, Webb P, Parsonnet J. H pylori and gastric cancer. *Lancet* 1994; **343**: 243-244
3 **Crew KD**, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol* 2006; **12**: 354-362
4 **Suerbaum S**, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; **347**: 1175-1186
5 **Malekzadeh R**, Sotoudeh M, Derakhshan MH, Mikaeli J, Yazdanbod A, Merat S, Yoonessi A, Tavangar M, Abedi BA, Sotoudehmanesh R, Pourshams A, Asgari AA, Doulatshahi S, Alizadeh BZ, Arshi S, Madjidpoor A, Mir Moomen S, Fleischer DE. Prevalence of gastric precancerous lesions in Ardabil, a high incidence province for gastric adenocarcinoma in the northwest of Iran. *J Clin Pathol* 2004; **57**: 37-42
6 **Singh V**, Trikha B, Nain CK, Singh K, Vaiphei K. Epidemiology of Helicobacter pylori and peptic ulcer in India. *J Gastroenterol Hepatol* 2002; **17**: 659-665
7 **Ayoola AE**, Ageely HM, Gadour MO, Pathak VP. Prevalence of Helicobacter pylori infection among patients with dyspepsia in South-Western Saudi Arabia. *Saudi Med J* 2004; **25**: 1433-1438
8 **Gunaid AA**, Hassan NA, Murray-Lyon I. Prevalence and risk factors for Helicobacter pylori infection among Yemeni dyspeptic patients. *Saudi Med J* 2003; **24**: 512-517
9 **Bani-Hani KE**, Hammouri SM. Prevalence of Helicobacter pylori in Northern Jordan. Endoscopy based study. *Saudi Med J* 2001; **22**: 843-847
10 **Zaitoun AM**. Histological study of chronic gastritis from the United Arab Emirates using the Sydney system of classification. *J Clin Pathol* 1994; **47**: 810-815
11 **Britt DP**, Barakat MH, Tungekar MF, Painchaud SM, Adlouni M, Kern K, Malhas L. Helicobacter pylori in dyspeptic patients in Kuwait. *J Clin Pathol* 1990; **43**: 987-991
12 **Alborzi A**, Soltani J, Pourabbas B, Oboodi B, Haghighat M, Hayati M, Rashidi M. Prevalence of Helicobacter pylori infection in children (south of Iran). *Diagn Microbiol Infect Dis* 2006; **54**: 259-261
13 **Massarrat S**, Saberi-Firoozi M, Soleimani A, Himmelmann GW, Hitzges M, Keshavarz H. Peptic ulcer disease, irritable bowel syndrome and constipation in two populations in Iran. *Eur J Gastroenterol Hepatol* 1995; **7**: 427-433
14 **Bafandeh Y**, Esmaeeli H, Aharizad S. Helicobacter pylori infection rates in duodenal ulcer patients in a population with high prevalence of infection. *Indian J Gastroenterol* 2005; **24**: 130
15 **Siavoshi F**, Malekzadeh R, Daneshmand M, Ashktorab H. Helicobacter pylori endemic and gastric disease. *Dig Dis Sci* 2005; **50**: 2075-2080
16 **Nassrolahei M**, Khalilian A. Seropositivity of antibodies against Helicobacter pylori and hepatitis A virus in Iran. *Ann Saudi Med* 2004; **24**: 61-64
17 **Frenck RW Jr**, Clemens J. Helicobacter in the developing world. *Microbes Infect* 2003; **5**: 705-713
18 **Perez-Perez GI**, Rothenbacher D, Brenner H. Epidemiology of Helicobacter pylori infection. *Helicobacter* 2004; **9** Suppl 1: 1-6
19 **Talley NJ**, Silverstein MD, Agréus L, Nyrén O, Sonnenberg A, Holtmann G. AGA technical review: evaluation of dyspepsia. American Gastroenterological Association. *Gastroenterology* 1998; **114**: 582-595
20 **Tovey FI**, Hobsley M. Is Helicobacter pylori the primary cause of duodenal ulceration? *J Gastroenterol Hepatol* 1999; **14**: 1053-1056
21 **Holcombe C**, Omotara BA, Eldridge J, Jones DM. H. pylori, the most common bacterial infection in Africa: a random serological study. *Am J Gastroenterol* 1992; **87**: 28-30
22 NIH Consensus Conference. Helicobacter pylori in peptic ulcer disease. NIH Consensus Development Panel on Helicobacter pylori in Peptic Ulcer Disease. *JAMA* 1994; **272**: 65-69
23 **Peura DA**, Graham DY. Helicobacter pylori: consensus reached: peptic ulcer is on the way to becoming an historic disease. *Am J Gastroenterol* 1994; **89**: 1137-1139
24 **Chan FK**, Leung WK. Peptic-ulcer disease. *Lancet* 2002; **360**: 933-941
25 **Weir RD**, Backett EM. Studies of the epidemiology of peptic ulcer in a rural community: prevalence and natural history of dyspepsia and peptic ulcer. *Gut* 1968; **9**: 75-83
26 **Khuroo MS**, Mahajan R, Zargar SA, Javid G, Munshi S. Prevalence of peptic ulcer in India: an endoscopic and epidemiological study in urban Kashmir. *Gut* 1989; **30**: 930-934
27 **Lee I**, Lee H, Kim M, Fukumoto M, Sawada S, Jakate S, Gould VE. Ethnic difference of Helicobacter pylori gastritis: Korean and Japanese gastritis is characterized by male- and antrum-predominant acute foveolitis in comparison with American gastritis. *World J Gastroenterol* 2005; **11**: 94-98
28 **Bernersen B**, Johnsen R, Straume B, Burhol PG, Jenssen TG, Stakkevold PA. Towards a true prevalence of peptic ulcer: the Sørreisa gastrointestinal disorder study. *Gut* 1990; **31**: 989-992
29 **Kurata JH**, Haile BM, Elashoff JD. Sex differences in peptic ulcer disease. *Gastroenterology* 1985; **88**: 96-100
30 **Satti MB**, Twum-Danso K, al-Freihi HM, Ibrahim EM, al-Gindan Y, al-Quorain A, al-Ghassab G, al-Hamdan A, al-Idrissi HY. Helicobacter pylori-associated upper gastrointestinal disease in Saudi Arabia: a pathologic evaluation of 298 endoscopic biopsies from 201 consecutive patients. *Am J Gastroenterol* 1990; **85**: 527-534
31 **Singh K**, Ghoshal UC. Causal role of Helicobacter pylori infection in gastric cancer: an Asian enigma. *World J Gastroenterol* 2006; **12**: 1346-1351
32 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789
33 **Sipponen P**, Hyvärinen H. Role of Helicobacter pylori in the pathogenesis of gastritis, peptic ulcer and gastric cancer. *Scand J Gastroenterol Suppl* 1993; **196**: 3-6
34 **Kuipers EJ**, Uyterlinde AM, Peña AS, Roosendaal R, Pals G, Nelis GF, Festen HP, Meuwissen SG. Long-term sequelae of Helicobacter pylori gastritis. *Lancet* 1995; **345**: 1525-1528
35 **Nardone G**, Rocco A, Staibano S, Mezza E, Autiero G, Compare D, De Rosa G, Budillon G. Diagnostic accuracy of the serum profile of gastric mucosa in relation to histological and morphometric diagnosis of atrophy. *Aliment Pharmacol Ther* 2005; **22**: 1139-1146

S- Editors Pan BR **L- Editor** Wang XL **E- Editor** Bi L

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5483-5489
World Journal of Gastroenterology ISSN 1007-9327

BASIC RESEARCH

Occurrence of cGMP/nitric oxide-sensitive store-operated calcium entry in fibroblasts and its effect on matrix metalloproteinase secretion

Yong Huang, Min-Qiang Lu, Hua Li, Chi Xu, Shu-Hong Yi, Gui-Hua Chen

Yong Huang, Min-Qiang Lu, Hua Li, Chi Xu, Shu-Hong Yi, Gui-Hua Chen, Liver Transplantation Centre, the Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510630, Guangdong Province, China
Supported by the Major State Basic Research Development Program (973 Program) of China, No.2003CB515507
Correspondence to: Dr. Gui-Hua Chen, Liver Transplantation Centre, the Third Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510630, Guangdong Province, China. chghzssy@yahoo.com.cn
Telephone: +86-20-87595523 Fax: +86-20-87595523
Received: 2006-04-19 Accepted: 2006-06-14

Abstract

AIM: To examine the existence of Nitric oxide/cGMP sensitive store-operated Ca^{2+} entry in mouse fibroblast NIH/3T3 cells and its influence on matrix metalloproteinase (MMP) production and adhesion ability of fibroblasts.

METHODS: NIH/3T3 cells were cultured. Confocal laser scanning microscopy was used to examine the existence of thapsigargin-induced store-operated Ca^{2+} entry in fibroblasts. Gelatin zymography and semiquantitative reverse transcriptase-polymerase chain reaction (RT-PCR) were employed to detect the involvement of $[Ca^{2+}]_i$ and NO/cGMP in MMP secretion. The involvement of NO/cGMP-sensitive Ca^{2+} entry in adhesion was determined using matrigel-coated culture plates.

RESULTS: 8-bromo-cGMP inhibited the thapsigargin-induced Ca^{2+} entry in 3T3 cells. The cGMP-induced inhibition was abolished by an inhibitor of protein kinase G, KT5823 (1μmol/L). A similar effect on the Ca^{2+} entry was observed in 3T3 cells in response to a NO donor, (±)-*S*-nitroso-*N*-acetylpenicillamine (SNAP). The inhibitory effect of SNAP on the thapsigargin-induced Ca^{2+} entry was also observed, indicating NO/cGMP-regulated Ca^{2+} entry in 3T3 cells. Results of gelatin zymography assay showed that addition of extracellular Ca^{2+} concentration induced MMP release and activation in a dose-dependent manner. RT-PCR also showed that cGMP and SNAP reduced the production of MMP mRNA in 3T3 cells. Experiments investigating adhesion potentials demonstrated that cGMP and SNAP could upgrade 3T3 cell attachment rate to the matrigel-coated culture plates.

CONCLUSION: NO/cGMP sensitive store-operated Ca^{2+} entry occurs in fibroblasts, and attenuates their adhesion potentials through its influence on MMP secretion.

Key words: cGMP; Nitric oxide; Protein kinase G; Store-operated Ca^{2+} entry; Matrix metalloproteinase; Fibroblast

Huang Y, Lu MQ, Li H, Xu C, Yi SH, Chen GH. Occurrence of cGMP/Nitric oxide-sensitive store-operated calcium entry in fibroblasts and its effect on matrix metalloproteinase secretion. *World J Gastroenterol* 2006; 12(34): 5483-5489

http://www.wjgnet.com/1007-9327/12/5483.asp

INTRODUCTION

Members of matrix metalloproteinase (MMP) family have been broadly implicated in both physiological and pathophysiological tissue remodeling[1], and play a key role in tumor invasion and metastasis. Since degradation of extracellular matrix (ECM) components by MMP is critical for tumor cell invasion and metastasis[2,3], MMP-2 degrades type Ⅳ collagen, a major component of the basement membrane because of its activity. Recent studies have revealed that tumor cells utilize MMP-2 produced by neighboring stromal cells including fibroblasts rather than by tumor cells themselves[4,5] for tumor progression, invasion, and metastasis, and that tumor cells can stimulate MMP production by stromal cells via soluble factors such as cytokines[6-9] or through cell-cell interaction mediated by cell adhesion molecules such as CD147[10,11].

Studies have proved that stromal cells mainly release MMP around tumor cells, and that calcium is a second intracellular messenger which mediates a wide range of cellular responses. These studies aroused our interest in whether calcium ions are involved in MMP secretion. In non-excitable cells, the influx of Ca^{2+} is a biphasic process, which consists of an initial transient phase followed by a large and sustained phase. Under Ca^{2+}-free conditions, the response is only a small and transient rise in $[Ca^{2+}]_i$, which should reflect the release of intracellular Ca^{2+} stores. When extracellular Ca^{2+} is reintroduced in the absence of the agonist, there is a large rise in $[Ca^{2+}]_i$. Since the depletion of intracellular Ca^{2+} stores is apparently the single

mechanism at work under such a circumstance, it has been hypothesized that the Ca^{2+} entry is activated by the depletion of Ca^{2+} stores and this Ca^{2+} entry is thus called store-operated Ca^{2+} entry (SOC)[12].

Nitric oxide (NO) is also known to inhibit Ca^{2+} entry through L-type Ca^{2+} channels (LTCC) in SMCs via cGMP-dependent mechanisms or via membrane hyperpolarization due to cGMP-dependent activation of Ca^{2+}-dependent K^+ channels. Besides the direct effects of NO on Ca^{2+} entry, it can also activate heme-containing soluble guanylyl cyclase (sGC) and catalyze the production of cGMP from GTP, thus playing a role in NO/cGMP/protein kinase G (PKG) pathway. Although many reports have described the regulating mechanisms on the calcium channels, they all focused on the end result-- increase in $[Ca^{2+}]_i$. Thus further investigation is certainly required to clarify the role of NO/cGMP in SOC and MMP secretion.

The aim of the present study was to demonstrate the existence of NO/cGMP-regulated SOC in NIH/3T3 cells and to further explore the influence of store-operated Ca^{2+} entry on MMP secretion and adhesion potential in NIH/3T3 cells.

MATERIALS AND METHODS

Cell culture and reagents

NIH/3T3 cells (CRL-1658, obtained from American Tissue Culture Collection, ATCC) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 100 mL/L fetal calf serum. Cultures were maintained at 37℃ in a humidified incubator under an atmosphere of 950 mL/L air and 50 mL/L CO_2. 8-bromo-cGMP, (±)-*S*-nitroso-*N*-acetylpenicillamine (SNAP), *N*-methyl-(8*R*,9*S*,11*S*)-(-)-9-methoxy-9-methoxycarbonyl-8-methyl-3,10-dihydro-8-11-epoxy-1H, 8H, 11H-2.7b, 11*a*-triazadibenzo(a,g)cycloocta(cde) trinden-1-one (KT5823), N^G-nitro-L-arginine methyl ester (L-NAME) and thapsigargin were obtained from Calbiochem (La Jolla, CA). DMEM and fetal bovine serum were purchased from Life Technologies, Inc. Fluo3/AM was obtained from Molecular Probes, Inc. (Eugene, OR). One Step RNA PCR kit (AMV) was purchased from TaKaRa Biotechnology (Dalian) Co., Ltd. MMP-2 primers (Accession no. NM008610; 5'ACCATCGAGACCATGCGG3', 5'CTCCCCCAACACCAGTGC3', 334 bp), MMP-9 primers (Accession no. NM013599; 5'TTCTGCCCTACCCGAGTGGA3', 5'CATAGTGGGAGGTGCTGTCGG3', 426 bp), β-actin primers (5'CTCACTGTCCACCTTCCAG3', 5'CGACCATCCTCCTCTTAGG3', 494 bp) were obtained from SaiBaiSheng Co. Trizol for total RNA isolation was from Life Technologies, Inc. Matrigel was obtained from Becton Dickinson Laboratory (Bedford, MA). Other reagents were from Sigma-Aldrich (St. Louis, MO).

Measurement of [Ca2+]i in single cells by confocal laser scanning microscopy

After an overnight attachment, the cells were rinsed in 0.01 mol/L PBS and loaded with 5 μmol/L Fluo3/AM for 45 min in dark at 37℃ in normal PBS (NPBS) containing 2 mmol/L $CaCl_2$, pH 7.4. The cells were then washed and resuspended in NPBS. To start the experiment, the cells were pretreated with 4 μmol/L thapsigargin for 2 min. Then the cells were washed with and maintained briefly in PBS containing no Ca^{2+} and 2 mmol/L EGTA. Unless stated otherwise, the cells were pretreated with or without chemicals (i.e. 8-bromo-cGMP, KT5823, SNAP, L-NAME or $NiCl_2$) for 1-2 min. The fluorescent signal of cytoplasmic calcium ion concentration in 3T3 cells was determined by fluorescence using Bio-Rad MRC 1024 visible light CLSM (Bio-Rad, Hercules, CA) and argon (488/526 nm) laser light.

Gelatin zymography

The conditioned media were collected by centrifugation, concentrated and dialyzed. The dialyzed samples containing an equal amount (20 μg) of total protein were mixed with the sample buffer, incubated in water bath (about 55℃) for 3-5 min and loaded onto the zymographic 100 g/L SDS-polyacrylamide gel containing gelatin (1g/L) and run under standard conditions (2 h at constant voltage of 100V). Afterwards, the gels were washed once with 50 mmol/L Tris (pH 7.4), containing 25 mL/L Triton X-100 for 30 min, and twice with 50 mmol/L Tris (pH 7.4). Gels were then incubated for 16-18 h in 50 mmol/L Tris (pH 7.5), 0.15 NaCl, 10 mmol/L $CaCl_2$, 1 mL/L Triton X-100 and 0.2 mg/L sodium azide. Finally, the gels were stained with Coomassie blue and washed with 75 mL/L acetic acid containing 200 mL/L methanol. The gels were subjected to densitometric analysis to quantitate the gelatinase activity by obtaining volumograms on a photo documentation system from UVItec (Cambridge, UK) using the UVIchrom acquisition software.

Semiquantitative reverse transcriptase-polymerase chain reaction

Total RNA was extracted from cGMP-pretreated 3T3 cells using Trizol agents. First-strand cDNA synthesis was performed using 1 μg of total RNA, 40 MU/L of RNase inhibitor, 20 MU/L sense and anti-sense primers of MMP-2, MMP-9 and β-actin, 10 mmol/L dNTP mixture, 25 mmol/L $MgCl_2$, 5 MU/L AMV Rtase XL, 5 MU/L AMV-optimized Taq. MMP-2, MMP-9 and β-actin primers were reported in Material section. According to One Step RNA PCR kit (AMV) instructions, the following conditions were used for MMP-2: 28 cycles of PCR amplification, denaturation at 94℃ for 1 min, annealing at 60℃ for 1 min, extension at 72℃ for 1 min; MMP-9: 30 cycles of PCR amplification, denaturation at 94℃ for 50 s, annealing at 60℃ for 1 min, and extension at 72℃ for 2 min; β-actin: 23 cycles of PCR amplification, denaturation at 94℃ for 30 s, annealing at 53℃ for 30 s, and extension at 72℃ for 1 min. Amplification was carried out in a GeneAmp 2400 PCR system (Perkins-Elmer, Foster City, CA). PCR products were resolved on 25 g/L agarose gel in the presence of 0.6 mg/L ethidium bromide. The intensities of the cDNA bands for each protein were normalized to β-actin band intensities. Images of the ethidium bromide (EB)-stained agarose gels were acquired with a digital Kodak camera (Eastman Kodak Company,

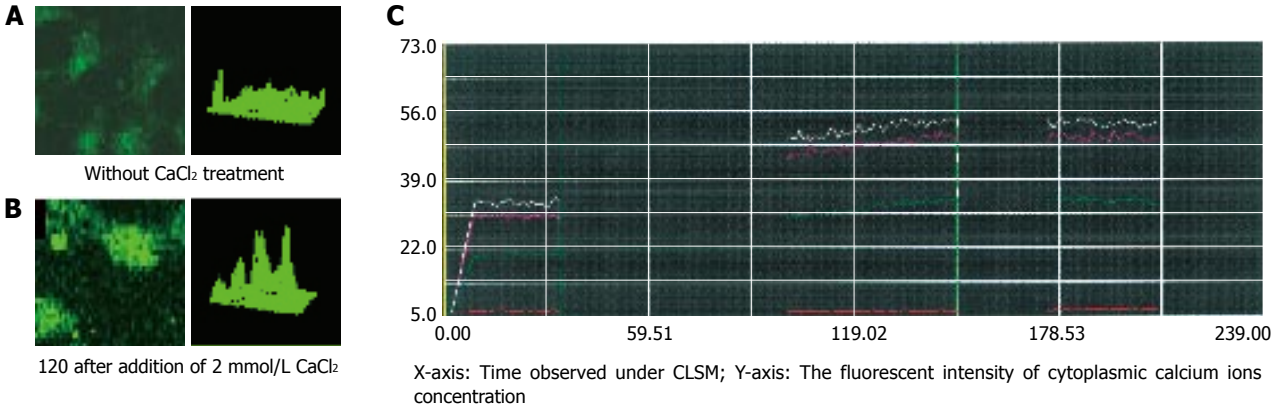


Figure 1 Tg-induced intracellular calcium ion concentration changes in 3T3 cells. **A:** The fluorescent intensity in 3T3 cells was 25-30 in Ca^{2+}-free and 2 mmol/L EGTA-containing medium after treatment with 4 μmol/L Tg; **B:** The fluorescent intensity rose to 50-55 in 3T3 cells after adding 2 mmol/L $CaCl_2$; **C:** Similar intracellular free Ca^{2+} concentration dynamic changes in 3T3 cells and Tg-induced elevation of $[Ca^{2+}]_i$ in 3T3 cells 120 min after treatment.

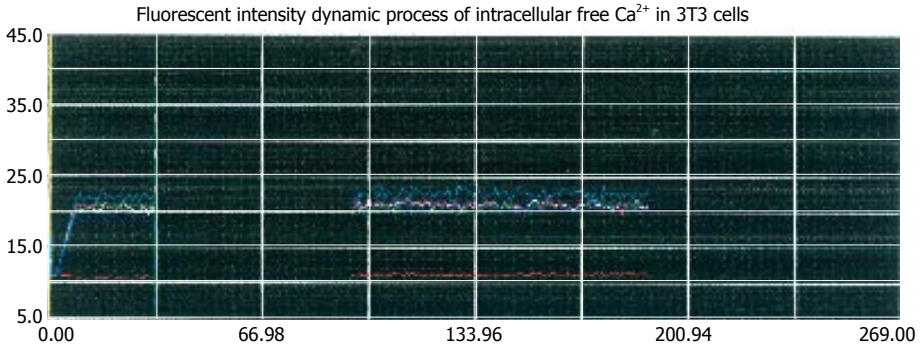


Figure 2 Inhibitory effect of Ni^{2+} on thapsigargin-induced rise in $[Ca^{2+}]_i$. The cells were incubated in NPBS with 4 μmol/L thapsigargin and 3 mmol/L $NiCl_2$ for 90 s, then the media were replaced with respective media containing 2 mmol/L $CaCl_2$ without EGTA, no significant change of $[Ca^{2+}]_i$ occurred after 120 s.

Rochester, NY, USA) and quantification of the bands was performed by imaging analysis software from Kodak.

Cell adhesion assay

The wells of 96-well culture plates were coated with matrigel at a concentration of 5 mg/L and incubated at 4℃ overnight. The coated wells were blocked with PBS containing 20 g/L bovine serum albumin for 30 min and then washed with PBS. Cell suspension in serum-free medium containing 1 g/L bovine serum albumin was added to the wells (2×10^4/well) and incubated at 37℃ in 50 mL/L CO_2 for 30-60 min with or without test agents (8-bromo-cGMP or SNAP). After the medium and nonattached cells were removed, 2 g/L crystal violet was added for 10 min. The plate was gently washed with tap water and dried in air for 24 h. Then 0.1mL of 50 g/L SDS with 500 mL/L ethanol was added for 20 min, the plate was read at 540 nm on an ELISA reader (Microplate Co., EL311SX).

Statistical analysis

Data were expressed as mean ± SD. Intracellular Ca^{2+} fluorescence ratio and percentages of attached cells were estimated with Student's *t*-test or analysis of variance followed by Student-Newman-Keuls test. $P < 0.05$ was considered statistically significant.

RESULTS

Thapsigargin-induced store-operated Ca^{2+} entry in fibroblast NIH/3T3 cells

Thapsigargin was used to deplete intracellular Ca^{2+} stores and induce Ca^{2+} entry from extracellular space. After treatment with 4 μmol/L thapsigargin in Ca^{2+}-free and 2 mmol/L EGTA-containing medium for 2 min, the addition of 2 mmol/L $CaCl_2$ induced a rise in $[Ca^{2+}]_i$ about 120 later (Figure 1). The thapsigargin-induced elevation of $[Ca^{2+}]_i$ was completely blocked by Ni^{2+} (3 mmol/L), a potent blocker of Ca^{2+} entry that competes for Ca^{2+}-binding sites in 3T3 cells (Figure 2), confirming the presence of capacitive Ca^{2+} entry in this cell type.

Effect of NO/cGMP/PKG signal pathway on store-operated Ca^{2+} entry

It has been reported that capacitive Ca^{2+} entry in hepatoma cells could be inhibited by cGMP, an activator of protein kinase G (PKG), and enhanced by KT5823, an inhibitor of PKG[13-15]. The present study examined the effect of cGMP and KT5823 on the capacitative Ca^{2+} entry in 3T3 cells. The $[Ca^{2+}]_i$ fluorescence ratios were obtained in cells without chemical treatment (control) or treated with 2 mmol/L cGMP or 1 μmol/L KT5823, respectively. Results showed that 2 mmol/L cGMP inhibited the Ca^{2+}

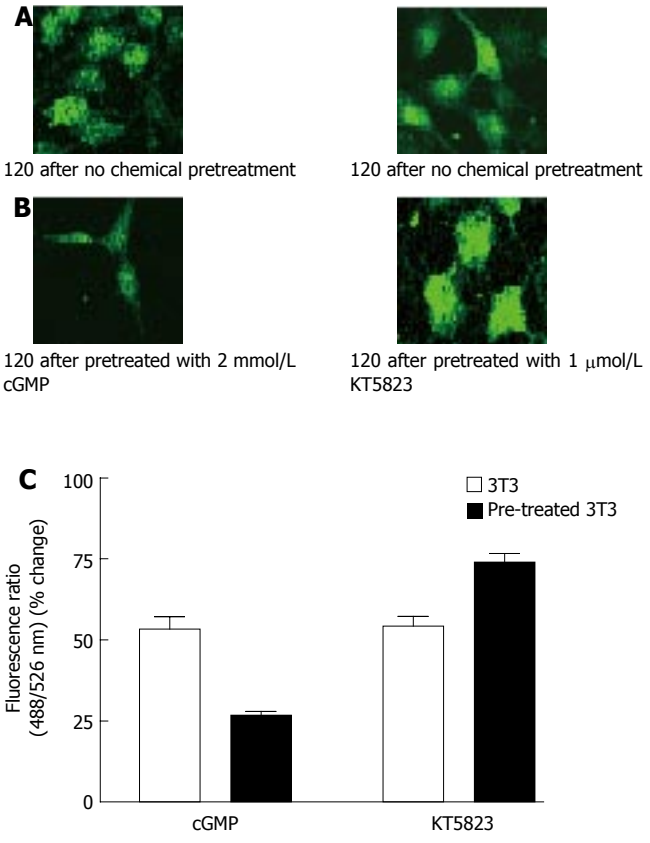


Figure 3 Effect of 8-bromo-cGMP and KT5823 on thapsigargin-induced Ca^{2+} influx. **A:** 3T3 cells were incubated in NPBS with 4 μmol/L thapsigargin for 2 min, then 2 mmol/L $CaCl_2$ was added into the medium; **B:** 3T3 cells were incubated in NPBS with 4 μmol/L thapsigargin for 2 min, 8-bromo-cGMP(2 mmol/L) or KT5823 (1 μmol/L) was introduced 1 min prior to the measurement, then 2 mmol/L $CaCl_2$ was added into the medium; **C:** Effects of cGMP and KT5823 on thapsigargin-induced Ca^{2+} influx.. The Ca^{2+} entry fluorescence ratios were obtained in cells without chemical treatment (control) or treated with 2 mmol/L 8-Br-cGMP (cGMP) or 1 μmol/L KT5823. The cells were incubated in NPBS with 4 μmol/L thapsigargin for 2 min prior to the chemical treatment. One mmol/L $CaCl_2$ was introduced to the medium to obtain the fluorescent signal of Ca^{2+} entry. The values are the mean ± SE (n = 4-6).

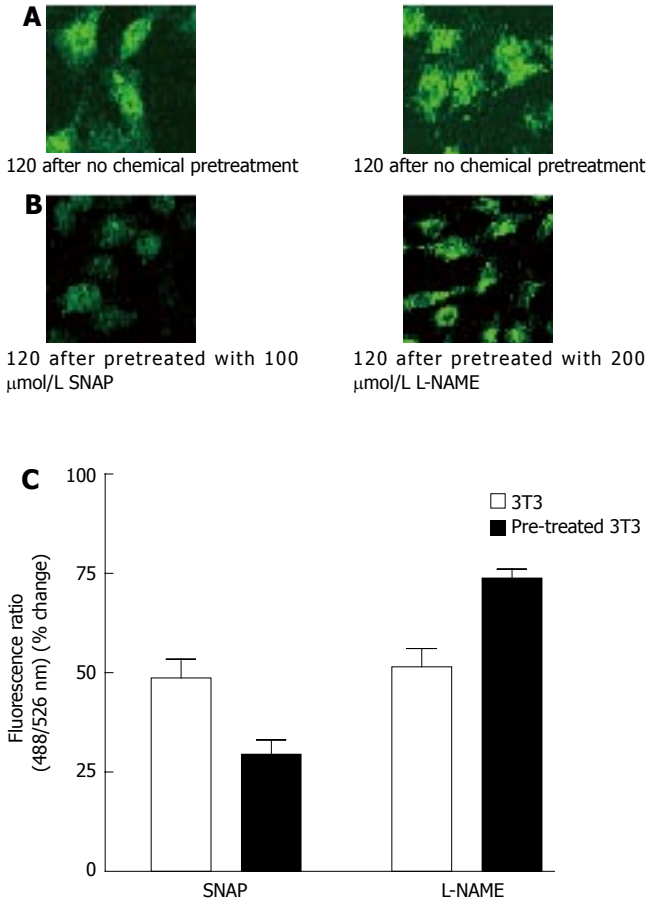


Figure 4 Effect of SNAP and L-NAME on thapsigargin-induced Ca^{2+} influx. **A:** 3T3 cells were incubated in NPBS with 4 μmol/L thapsigargin for 2 min, then 2 mmol/L $CaCl_2$ was added into the medium; **B**: 3T3 cells were incubated in NPBS with 4 μmol/L thapsigargin for 2 min, 100 μmol/L SNAP or 200 μmol/L L-NAME was introduced 1 min prior to the measurement, then 2 mmol/L $CaCl_2$ was added into the medium; **C:** Ca^{2+} entry fluorescence ratios were obtained in cells without chemical treatment (control) or treated with 100 μmol/L SNAP or 200 μmol/L L-NAME. The values are the mean ± SE (n = 4-6).

entry with 25.3% ± 1.3% inhibition compared with 3T3 cells not pretreated (53.0% ± 4.4%) ($P < 0.05$, Figure 3C). KT5823 (1 μmmol/L) stimulated the Ca^{2+} entry by 72.3% ± 3.9% ($P < 0.05$, Figure 3C).

NO is an important intracellular signal molecule that activates soluble guanylye cyclase to synthesize cGMP[16]. The present study was carried out to investigate the involvement of NO in regulation of the capacitive Ca^{2+} entry using a NO donor (SNAP) and L-NAME (a specific NOS inhibitor) to trigger production of endogenous cGMP. Results showed that SNAP also inhibited the thapsigargin-indued Ca^{2+} influx, 26.7% ± 6.4% inhibition was observed at 100 μmol/L SNAP compared with control (48.1% ± 5.2%) ($P < 0.05$, Figure 4C). L-NAME (200 μmol/L) could excite the thapsigargin-indued Ca^{2+} influx in 3T3 cells too, 71.6% ± 3.9% stimulation was observed in 3T3 cells compared with control (49.6% ± 5.8%) ($P < 0.05$, Figure 4C).

Effect of SOC on MMP mRNA expression

The expression and release of MMPs (such as MMP-2 and MMP-9) work through kinase signaling pathways[17-19], like NO-cGMP-Ca^{2+} signaling pathway[20-22]. The present study tested the involvement of SOC in regulating MMP release *in vitro*. RT-PCR showed that synthesis of MMP-2 and MMP-9 mRNA in cGMP-pretreated 3T3 cells was less than that in 3T3 cells not pretreated. Gelatin zymography showed that production of MMP-2 and MMP-9 in 3T3 medium culture was more than that in cGMP- pretreated 3T3 medium culture (Figure 5).

Involvement of $[Ca^{2+}]_i$ and NO/cGMP in MMP secretion

Zymography results showed that the increasing extracellular calcium concentrations (0, 0.09, 0.4, 0.8, 1.2 mmo/L) enhanced MMP-2 and MMP-9 secretion in a dose-dependent manner (Figure 6). In the present study, 4 μmol/L thapsigargin was used to deplete the intracellular Ca^{2+} store in order to induce Ca^{2+} entry. Zymography results showed pretreatment with 8-bromo-cGMP (2 mmol/L) or SNAP (200 μmol/L) significantly reduced the production of MMP-2 and MMP-9 in 3T3 cells, and 1 μmol/L KT5823 or 100 μmol/L L-NAME enhanced the production of MMP-2 and MMP-9 in 3T3 cells. These results indicated that NO/cGMP sensitive SOC was

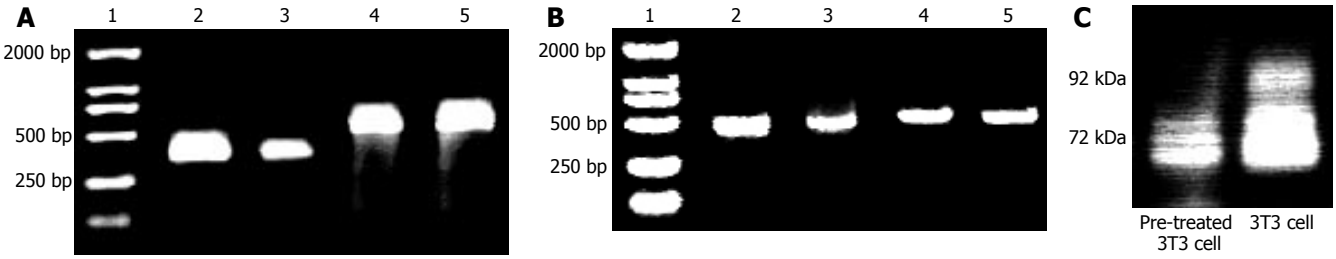


Figure 5 Expression of MMP in pretreated and not pretreated 3T3 cells. **A:** RT-PCR results of MMP-2 mRNA in both cells (1: DL2000 marker; 2: MMP-2 cDNA of 3T3 cells; 3: MMP-2 cDNA of 8-Br-cGMP (2 mmol/L) pre-treated 3T3 cells; 4: β-actin cDNA of 3T3 cells; 5: β-actin cDNA of pre-treated 3T3 cells); **B:** RT-PCR results of MMP-9 mRNA in pretreated and not pretreated 3T3 cells (1: DL2000 marker; 2: MMP-9 cDNA of 3T3 cells; 3: MMP-9 cDNA of 8-Br-cGMP (2 mM) pre-treated 3T3cells; 4: β-actin cDNA of 3T3 cells; 5: β-actin cDNA of pre-treated 3T3 cells); **C:** Gelatin zymography showing constitutive secretion of MMP-2 (72 kDa) and MMP-9 (92 kDa) into serum-free media by pretreated and not pretreated 3T3 cells.

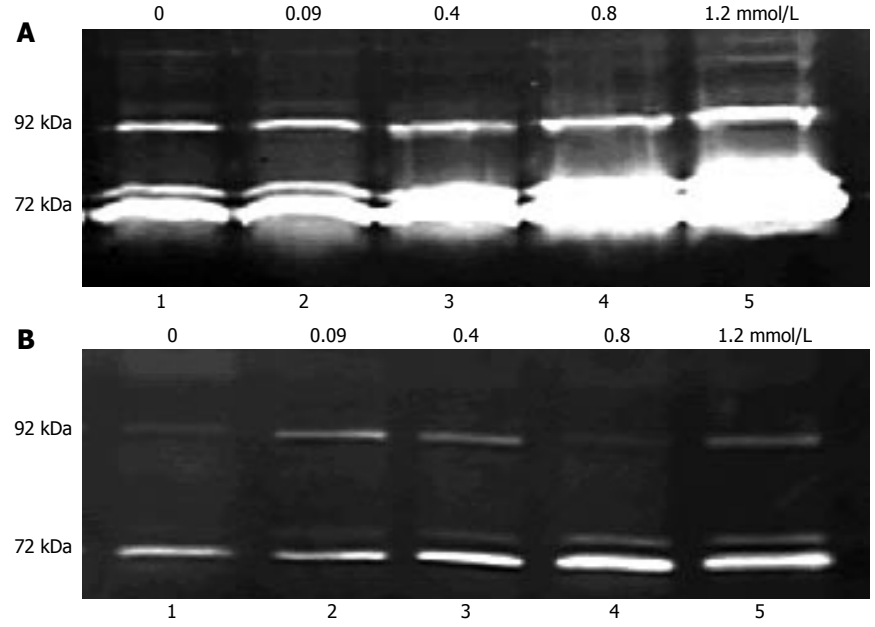


Figure 6 Dose-dependent effect of extracellular Ca^{2+} on MMP release. 8-bromo-cGMP (2 mmol/L) pre-treated and not pretreated 3T3 cells were plated in media with serial doses of Ca^{2+} (0, 0.09 ,0.4, 0.8, 1.2 mmol/L) and incubated for 16-18 h, 200 μL conditioned media was used as sample. The 72 kDa gelatinolytic bands corresponded to the MMP-2, the 92 kDa band to MMP-9. **A:** 3T3 cell mean ratio of 72 kDa in groups 1-5 was 0.198 ± 0.094, 0.287 ± 0.114, 0.486 ± 0.079, 1.247 ± 0.089 and 2.487 ± 0.179, respectively (n = 4-6); **B:** 8-bromo-cGMP (2 mmol/L) pre-treated 3T3 cell mean ratio of 72 kDa in groups 1-5 was 0.104 ± 0.084, 0.126 ± 0.086, 0.257 ± 0.102, 0.490 ± 0.173 and 0.784 ± 0.187, respectively (n = 4-6).

involved in MMP secretion of fibroblasts (Figure 7).

Involvement of NO/cGMP-sensitive Ca^{2+} entry in adhesion

We used matrigel-coated plates as models for investigation of possible signaling pathways involved in extracellular matrix-induced metastasis. Matrigel is a soluble basement membrane preparation containing almost all extracellular matrix components[23]. The present study was carried out with matrigel as an adhesion substratum. A significant difference in the amount of cells attached to the matrigel-coated plates was observed in 3T3 cells pre-treated and not pre-treated with cGMP (2 mmol/L) for 30 min (71.4% ± 0.5% *vs* 44.7% ± 2.5%) ($P < 0.05$). Pre-treatment with SNAP (200 μmol/L) showed that the percent of attached and non-attached 3T3 cells was 64.7% ± 1.2% and 46.3% ± 2.0%, respectively ($P < 0.05$, Figure 8).

DISCUSSION

Collagenolytic degradation of endothelial and parenchymal basement membranes is a necessary step in the process of tumor invasion and angiogenesis. Neutral MMP-2 and MMP-9 mainly secreted locally by stromal cells, degrade the basement membrane and extracellular matrix collagens. However, little is known about the signaling pathways that regulate the production of these enzymes. Previous studies have demonstrated that transforming growth factor-β1 (TGF-β1) plays a role in transmembrane signal transduction of MMP-2 regulation[24]. The present study described a role of calcium mobilization in the regulation of MMP expression.

The present study demonstrated that the existence of NO/cGMP-sensitive store-operated Ca^{2+} entry in mouse fibroblast NIH/3T3 cells, which is allergic to NO/cGMP. The thapsigargin-induced SOC appears to be evidenced by the magnitude of the Ca^{2+} entry and its blockage by Ni^{2+}, cGMP or SNAP inhibits the thapsigargin-induced Ca^{2+} entry in 3T3 cells and can be reversed by KT5823 (highly specific PKG inhibitor), indicating a negative regulatory mechanism involving NO/cGMP *via* a PKG-dependent pathway.

Characterization of the regulation of interstitial collagenase (MMP-1) and stromelysidtransin (MMP-3) indicates that there are at least two known regulatory arms for the secretion of these MMPs. Treatment with TGF-β1 inhibits the production of these MMP genes through TIE, a novel CIS-acting transcriptional element[25,26], whereas phorbol ester-mediated stimulation of transcription is

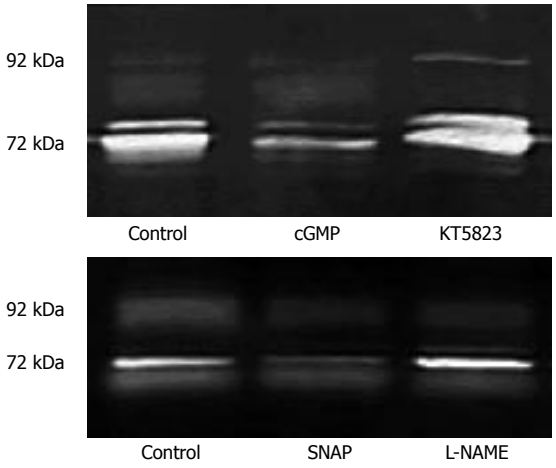


Figure 7 cGMP/KT5823 and SNAP/L-NAME affecting MMP release 3T3 cells via Tg-induced SOC. 3T3 cells were incubated in NPBS with 4 μmol/L thapsigargin for 2 min. Each kind of chemical reagents (2 mmol/L cGMP, 1 μmol/L KT5823, 100 μmol/L SNAP or 200 μmol/L L-NAME) was introduced 1 min prior to the measurement, then 2 mmol/L $CaCl_2$ was added into each medium and incubated for 16-18 h. The 72 kDa gelatinolytic band (MMP-2) was obtained in cells without chemical treatment (control) or treated with cGMP, KT5823, SNAP, L-NAME.

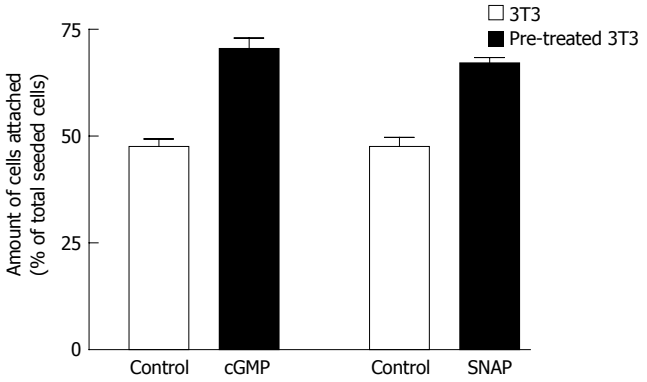


Figure 8 Effects of cGMP or SNAP on adhesion potentials of 3T3 cells to matrigel by adhesion assay. 3T3 cells pretreated with 4 μmol/L Tg were suspended in serum-free medium supplemented with no chemical reagent (control) or cGMP (2 mmol/L) or SNAP (200 μmol/L), 2 mmol/L $CaCl_2$ was added to induce SOC, then seeded into the matrigel (5 mg/L)-coated wells. After incubation for 30 min at 37℃, the percentage of adhered cells was determined using colorimetric crystal violet assay. The values are the mean ± SE (n = 6-8).

through phorbol ester-responsive elements (TREs) and AP-1 sites[27,28]. Regulation of matrix metalloproteinases has also been linked to the second cAMP messenger signal transduction pathway[29,30]. Several investigators have demonstrated the induction of MMP-1 production in response to cAMP either directly or after cell stimulation with cytokines that signal their effects through cAMP[29,31]. However, in contrast to MMP-1 and MMP-3, neither MMP-2 nor MMP-9 has TIE or cAMP-regulated elements defined in their 5' sequences[27,32]. The TRE mechanism has been implicated in the regulation of MMP-9 but not MMP-2[27]. AP-1 sites have not been documented for MMP-2, although AP-1 and AP-2 sites are in the upstream of MMP-9[27,32,33]. It was reported that there is a NF-κB binding site in the upstream sequence of MMP-9[34]. Besides the factors influencing MMP secretion, calcium-activated signal transduction steps are also a common thread underlying important signal transduction pathways, including protein kinase C, phosphoinositide metabolism, and generation of arachidonic acid. However, few calcium-mediated transcriptional pathways have been identified for MMP secretion.

The present study was to identify NO/cGMP-sensitive production and activation of MMP through SOC. In our study, adding extracellular Ca^{2+} concentration induced release and activation of MMP (MMP-2, MMP-9) in a dose-dependent manner, cGMP and SNAP reduced MMP secretion in 3T3 cells. RT-PCR displayed that MMP-2 and MMP-9 mRNA synthesis in cGMP- pretreated 3T3 cells was significantly less than that in non cGMP-pretreated 3T3 cells. Tumor invasion is greatly dependent on the permissive action of the microenvironment. One critical factor is the production of proteolytic enzymes involved in the degradation and remodeling of ECM. Among these enzymes, MMPs represent a large family playing a key role in cell proliferation, angiogenesis, tumor invasion and metastasis[6]. These enzymes principally degrade the ECM components and attenuate cell-cell adhesion ability so as to promote tumor invasion and metastasis.

Our experiments investigating adhesion potentials demonstrated that the attachment rate of 3T3 cells living in high extracellular Ca^{2+} concentration of the matrigel-coated culture plates was lower than that in low extracellular Ca^{2+} concentration of the matrigel-coated culture plates, suggesting that increased cellular Ca^{2+} concentration attenuates the adhesion potentials in fibroblasts by enhancing MMP secretion. The attachment rate could be upgraded by cGMP and SNAP in 3T3 cells, indicating that interference with NO/cGMP/PKG pathway sensitivity of fibroblasts reduces SOC and inhibits MMP secretion at a considerable level, thus strengthening the adhesion potential of fibroblasts and decreasing the chance of HCC metastasis.

In conclusion, store-operated Ca^{2+} channels exist in mouse fibroblast cells and are involved in MMP secretion. NO/cGMP/PKG pathway negatively regulates this Ca^{2+} entry. Increased extracellular Ca^{2+} concentration may promote degradation of ECM components by increasing MMP secretion and attenuating fibroblast adhesion potential.

REFERENCES

1 **Woessner JF Jr**. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J* 1991; **5**: 2145-2154
2 **Gauthier MC**, Racine C, Ferland C, Flamand N, Chakir J, Tremblay GM, Laviolette M. Expression of membrane type-4 matrix metalloproteinase (metalloproteinase-17) by human eosinophils. *Int J Biochem Cell Biol* 2003; **35**: 1667-1673
3 **Matsuo Y**, Hashimoto S, Koga T, Yonemitsu Y, Yoshino I, Sugimachi K, Honda H, Masuda K, Sueishi K. Growth pattern correlates with the distribution of basement membrane and prognosis in lung adenocarcinoma. *Pathol Res Pract* 2004; **200**: 517-529
4 **Kim JR**, Kim CH. Association of a high activity of matrix metalloproteinase-9 to low levels of tissue inhibitors of metalloproteinase-1 and -3 in human hepatitis B-viral hepatoma cells. *Int J Biochem Cell Biol* 2004; **36**: 2293-2306
5 **Nomura H**, Sato H, Seiki M, Mai M, Okada Y. Expression of membrane-type matrix metalloproteinase in human gastric carcinomas. *Cancer Res* 1995; **55**: 3263-3266
6 **Klein G**, Vellenga E, Fraaije MW, Kamps WA, de Bont ES.

The possible role of matrix metalloproteinase (MMP)-2 and MMP-9 in cancer, e.g. acute leukemia. *Crit Rev Oncol Hematol* 2004; **50**: 87-100

7 **Lohi J**, Lehti K, Westermarck J, Kähäri VM, Keski-Oja J. Regulation of membrane-type matrix metalloproteinase-1 expression by growth factors and phorbol 12-myristate 13-acetate. *Eur J Biochem* 1996; **239**: 239-247

8 **Okada A**, Bellocq JP, Rouyer N, Chenard MP, Rio MC, Chambon P, Basset P. Membrane-type matrix metalloproteinase (MT-MMP) gene is expressed in stromal cells of human colon, breast, and head and neck carcinomas. *Proc Natl Acad Sci USA* 1995; **92**: 2730-2734

9 **Simi L**, Andreani M, Davini F, Janni A, Pazzagli M, Serio M, Orlando C. Simultaneous measurement of MMP9 and TIMP1 mRNA in human non small cell lung cancers by multiplex real time RT-PCR. *Lung Cancer* 2004; **45**: 171-179

10 **Suzuki S**, Sato M, Senoo H, Ishikawa K. Direct cell-cell interaction enhances pro-MMP-2 production and activation in co-culture of laryngeal cancer cells and fibroblasts: involvement of EMMPRIN and MT1-MMP. *Exp Cell Res* 2004; **293**: 259-266

11 **Sameshima T**, Nabeshima K, Toole BP, Yokogami K, Okada Y, Goya T, Koono M, Wakisaka S. Glioma cell extracellular matrix metalloproteinase inducer (EMMPRIN) (CD147) stimulates production of membrane-type matrix metalloproteinases and activated gelatinase A in co-cultures with brain-derived fibroblasts. *Cancer Lett* 2000; **157**: 177-184

12 **Tiruppathi C**, Minshall RD, Paria BC, Vogel SM, Malik AB. Role of Ca2+ signaling in the regulation of endothelial permeability. *Vascul Pharmacol* 2002; **39**: 173-185

13 **Jiang JL**, Zhou Q, Yu MK, Ho LS, Chen ZN, Chan HC. The involvement of HAb18G/CD147 in regulation of store-operated calcium entry and metastasis of human hepatoma cells. *J Biol Chem* 2001; **276**: 46870-46877

14 **Xu X**, Star RA, Tortorici G, Muallem S. Depletion of intracellular Ca2+ stores activates nitric-oxide synthase to generate cGMP and regulate Ca2+ influx. *J Biol Chem* 1994; **269**: 12645-12653

15 **Yao X**, Kwan HY, Chan FL, Chan NW, Huang Y. A protein kinase G-sensitive channel mediates flow-induced Ca(2+) entry into vascular endothelial cells. *FASEB J* 2000; **14**: 932-938

16 **Denninger JW**, Marletta MA. Guanylate cyclase and the NO/cGMP signaling pathway. *Biochim Biophys Acta* 1999; **1411**: 334-350

17 **Lee SD**, Wu CC, Chang YC, Chang SH, Wu CH, Wu JP, Hwang JM, Kuo WW, Liu JY, Huang CY. Porphyromonas gingivalis-induced cellular hypertrophy and MMP-9 activity via different signaling pathways in H9c2 cardiomyoblast cells. *J Periodontol* 2006; **77**: 684-691

18 **Lim M**, Martinez T, Jablons D, Cameron R, Guo H, Toole B, Li JD, Basbaum C. Tumor-derived EMMPRIN (extracellular matrix metalloproteinase inducer) stimulates collagenase transcription through MAPK p38. *FEBS Lett* 1998; **441**: 88-92

19 **Tang W**, Hemler ME. Caveolin-1 regulates matrix metalloproteinases-1 induction and CD147/EMMPRIN cell surface clustering. *J Biol Chem* 2004; **279**: 11112-11118

20 **Eto M**, Bock R, Brautigan DL, Linden DJ. Cerebellar long-term synaptic depression requires PKC-mediated activation of CPI-17, a myosin/moesin phosphatase inhibitor. *Neuron* 2002; **36**: 1145-1158

21 **Goette A**, Lendeckel U, Klein HU. Signal transduction systems and atrial fibrillation. *Cardiovasc Res* 2002; **54**: 247-258

22 **Jurasz P**, Sawicki G, Duszyk M, Sawicka J, Miranda C, Mayers I, Radomski MW. Matrix metalloproteinase 2 in tumor cell-induced platelet aggregation: regulation by nitric oxide. *Cancer Res* 2001; **61**: 376-382

23 **Kleinman HK**, McGarvey ML, Hassell JR, Star VL, Cannon FB, Laurie GW, Martin GR. Basement membrane complexes with biological activity. *Biochemistry* 1986; **25**: 312-318

24 **Saed GM**, Zhang W, Diamond MP. Effect of hypoxia on stimulatory effect of TGF-beta 1 on MMP-2 and MMP-9 activities in mouse fibroblasts. *J Soc Gynecol Investig* 2000; **7**: 348-354

25 **Edwards DR**, Leco KJ, Beaudry PP, Atadja PW, Veillette C, Riabowol KT. Differential effects of transforming growth factor-beta 1 on the expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in young and old human fibroblasts. *Exp Gerontol* 1996; **31**: 207-223

26 **White LA**, Mitchell TI, Brinckerhoff CE. Transforming growth factor beta inhibitory element in the rabbit matrix metalloproteinase-1 (collagenase-1) gene functions as a repressor of constitutive transcription. *Biochim Biophys Acta* 2000; **1490**: 259-268

27 **Benbow U**, Brinckerhoff CE. The AP-1 site and MMP gene regulation: what is all the fuss about? *Matrix Biol* 1997; **15**: 519-526

28 **Westermarck J**, Kähäri VM. Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J* 1999; **13**: 781-792

29 **Van den Steen PE**, Dubois B, Nelissen I, Rudd PM, Dwek RA, Opdenakker G. Biochemistry and molecular biology of gelatinase B or matrix metalloproteinase-9 (MMP-9). *Crit Rev Biochem Mol Biol* 2002; **37**: 375-536

30 **Duca L**, Debelle L, Debret R, Antonicelli F, Hornebeck W, Haye B. The elastin peptides-mediated induction of pro-collagenase-1 production by human fibroblasts involves activation of MEK/ERK pathway via PKA- and PI(3)K-dependent signaling. *FEBS Lett* 2002; **524**: 193-198

31 **Cornwell TL**, Arnold E, Boerth NJ, Lincoln TM. Inhibition of smooth muscle cell growth by nitric oxide and activation of cAMP-dependent protein kinase by cGMP. *Am J Physiol* 1994; **267**: C1405-C1413

32 **McCawley LJ**, Li S, Benavidez M, Halbleib J, Wattenberg EV, Hudson LG. Elevation of intracellular cAMP inhibits growth factor-mediated matrix metalloproteinase-9 induction and keratinocyte migration. *Mol Pharmacol* 2000; **58**: 145-151

33 **Pan MR**, Chuang LY, Hung WC. Non-steroidal anti-inflammatory drugs inhibit matrix metalloproteinase-2 expression via repression of transcription in lung cancer cells. *FEBS Lett* 2001; **508**: 365-368

34 **Takahra T**, Smart DE, Oakley F, Mann DA. Induction of myofibroblast MMP-9 transcription in three-dimensional collagen I gel cultures: regulation by NF-kappaB, AP-1 and Sp1. *Int J Biochem Cell Biol* 2004; **36**: 353-363

S- Editor Pan BR **L- Editor** Wang XL **E- Editor** Liu WF

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5490-5494
World Journal of Gastroenterology ISSN 1007-9327

CLINICAL RESEARCH

Adiponectin and its receptors in rodent models of fatty liver disease and liver cirrhosis

Markus Neumeier, Claus Hellerbrand, Erwin Gäbele, Roland Buettner, Cornelius Bollheimer, Johanna Weigert, Andreas Schäffler, Thomas S Weiss, Monika Lichtenauer, Jürgen Schölmerich, Christa Buechler

Markus Neumeier, Claus Hellerbrand, Erwin Gäbele, Roland Buettner, Cornelius Bollheimer, Johanna Weigert, Andreas Schäffler, Jürgen Schölmerich, Christa Buechler, Department of Internal Medicine I, University of Regensburg, D-93042 Regensburg, Germany
Thomas S Weiss, Monika Lichtenauer, Department of Surgery, Center for Liver Cell Research, University of Regensburg, Germany
Supported by a grant from the Deutsche Forschungsgemeinschaft (BU 1141/3-2)
Correspondence to: Dr. Christa Buechler, PhD, Department of Internal Medicine I, University of Regensburg, Regensburg D-93042, Germany. christa.buechler@klinik.uni-regensburg.de
Telephone: +49-941-9447018 Fax: +49-941-9447019
Received: 2005-10-13 Accepted: 2006-03-10

Abstract

AIM: To determine circulating and hepatic adiponectin in rodents with fatty liver disease or liver cirrhosis and investigate expression of the adiponectin receptors AdipoR1 on the mRNA and protein level and AdipoR2 on the mRNA level.

METHODS: Fat fed rats were used as a model for fatty liver disease and bile duct ligation in mice to investigate cirrhotic liver. Expression of AdipoR1 and AdipoR2 mRNA was determined by real time RT-PCR. AdipoR1 protein was analysed by immunoblot. Adiponectin was measured by ELISA.

RESULTS: Systemic adiponectin is reduced in fat-fed rats but is elevated in mice after bile duct ligation (BDL). Hepatic adiponectin protein is lower in steatotic liver but not in the liver of BDL-mice when compared to controls. Adiponectin mRNA was not detected in human liver samples or primary human hepatocytes nor in rat liver but recombinant adiponectin is taken up by isolated hepatocytes *in-vitro*. AdipoR1 mRNA and AdipoR1 protein levels are similar in the liver tissue of control and fat fed animals whereas AdipoR2 mRNA is induced. AdipoR2 mRNA and AdipoR1 mRNA and protein is suppressed in the liver of BDL-mice.

CONCLUSION: Our studies show reduced circulating adiponectin in a rat model of fatty liver disease whereas circulating adiponectin is elevated in a mouse model of cirrhosis and similar findings have been described in humans. Diminished hepatic expression of adiponectin receptors was only found in liver cirrhosis.

Key words: Hepatic steatosis; Adiponectin; Liver cirrhosis; Adiponectin receptor 1; Adiponectin receptor 2

Neumeier M, Hellerbrand C, Gäbele E, Buettner R, Bollheimer C, Weigert J, Schäffler A, Weiss TS, Lichtenauer M, Schölmerich J, Buechler C. Adiponectin and its receptors in rodent models of fatty liver disease and liver cirrhosis. *World J Gastroenterol* 2006; 12(34): 5490-5494

http://www.wjgnet.com/1007-9327/12/5490.asp

INTRODUCTION

Obesity and especially visceral fat accumulation cause insulin resistance, a common risk factor for hepatic steatosis. Fatty liver is thought to represent the first step towards the subsequent development of liver fibrosis. Impaired mitochondrial function provides the second hit and promotes the generation of reactive oxygen species, which promote lipid peroxidation, the release of inflammatory cytokines, death of hepatocytes and activation of hepatic stellate cells. Non-alcoholic steatohepatitis (NASH) is a progressive disorder that can lead to liver cirrhosis and even hepatocellular carcinoma[1].

Adiponectin is highly abundant in human serum and is secreted by adipose tissue in inverse proportion to the body mass index[2]. Adiponectin improves whole body insulin sensitivity and in addition exerts anti-inflammatory effects by reducing NFκB activation[3]. Low adiponectin levels are associated with NASH independent of insulin resistance and body mass index indicating a protective effect for adiponectin in liver disease[4]. This idea is supported by studies in rodents where recombinant adiponectin given to leptin-deficient ob/ob mice ameliorates hepatic steatosis and normalizes alanine aminotransferase levels[5]. Besides these protective effects on fatty liver disease adiponectin attenuates T-cell mediated hepatic inflammation by reducing the release of proinflammatory cytokines, the activation of hepatic stellate cells and cell death of hepatocytes[6].

Two 7-transmembrane proteins, AdipoR1 and AdipoR2, have been identified to function as adiponectin receptors[7]. AdipoR1 mRNA is mainly expressed in the

human heart and skeletal muscle, whereas AdipoR2 was supposed to be the main receptor in the liver[7]. Recently a prominent protein expression of AdipoR1 in primary hepatocytes was demonstrated indicating that AdipoR1 may also be important in hepatic signal transduction[8]. Adiponectin activates the AMP-activated protein kinase (AMPK) and PPARα[7] but may also inhibit the binding of growth factors to their corresponding receptors independent of AdipoR1 and AdipoR2[9].

Although there is a well documented relationship between low adiponectin and liver disease, the role of adiponectin receptors is less clear[4,6]. In addition, mainly AdipoR2 has been analysed with regard to liver function[10,11]. Therefore the expression of AdipoR1 was investigated in rodent models of fatty liver disease and liver cirrhosis.

MATERIALS AND METHODS

Subjects

Wistar rats were fed a standard rodent chow (6 animals) or a high fat diet for twelve weeks (six animals) as recently described[12]. Male C57Bl/6J mice (body weight 25-30 g) underwent common bile duct ligation (BDL) and transection as previously reported[13]. Another group of animals was sham-operated to serve as a control. For analysis of adiponectin or AdipoR1 protein four control and five BDL mice were used. Analysis of mRNA expression was performed with total RNA isolated from the liver of eight control and four BDL animals. All animal procedures were performed under the guidelines set by The University of Regensburg Institutional Animal Care and Use Committee. Primary hepatocytes from three different donors were isolated and cultivated as described before[14]. Tissue samples from human liver resection were obtained from patients undergoing partial hepatectomy. Experimental procedures were performed according to the guidelines of the charitable state controlled foundation HTCR (Human Tissue and Cell Research), with the informed patient's consent[15] approved by the local ethical committee of the University of Regensburg.

Culture media and reagents

RPMI medium was from Biochrom (Southborough, MA, USA), RNeasy Mini Kit from Qiagen (Hilden, Germany) and oligonucleotides were synthesized by Metabion (Planegg-Martinsried, Germany). LightCycler FastStart DNA Master SYBR Green I was purchased from Roche (Mannheim, Germany). AdipoR1 peptide antibody was raised as recently described[8]. AdipoR2 protein was not analysed because several antibodies investigated did not specifically detect in-vitro translated AdipoR2 by immunoblot (own unpublished results).

ELISAs for human and mouse adiponectin and human recombinant adiponectin were from R&D systems (Wiesbaden-Nordenstadt, Germany). ELISAs for rat adiponectin from BioCat (Heidelberg, Germany). PI3-Kinase p85 antibody was from Upstate (Lake Placid, NY, USA). Recombinant human leptin was from Sigma Chemical (Deisenhofen, Germany) and 100 μg/L were used.

Monitoring of gene expression by real-time RT-PCR

Real-time RT-PCR was performed as recently described[16]. The primers are listed in Table 1. Amplification in the LightCycler capillaries was done for 45 cycles with initial incubation of ten minutes at 95℃ for activation of Taq polymerase. Cycling parameters were 15 s at 95℃, ten seconds 60℃ and ten seconds at 72℃. The second derivative method was used for quantification with the LightCycler software. For quantification of the results the standard curve method was used. Normalization was performed by dividing each value calculated for a specific gene by the value of the corresponding housekeeping gene. QuantiTect Primer Assays (Qiagen) for use in real-time RT-PCR with SYBR Green detection were used to determine rat adiponectin mRNA.

Table 1 Oligonucleotides used for real-time RT-PCR

Name	Sequence
AdipoR1 uni (h)	5´-GGGGAATTCTCTTCCCACAAAGGATCTGTG GTG-3´
AdipoR1 rev (h)	5´-GGGCTGCAGTTAAGTTTCTGTATGAATGCG GAAGAT-3´
AdipoR2 uni (h, m)	5´-GGGGAATTCAACGAGCCAACAGAAAACCG ATTG-3´
AdipoR2 rev (h, m)	5´-GGGCTGCAGCTAAATGTTGCCTGTTTCTGTG TGTAT-3´
β-actin uni (h)	5'-CTACGTCGCCCTGGACTTCGAGC-3´
β-actin rev (h)	5'-GATGGAGCCGCCGATCCACACGG-3´
AdipoR1 uni (m)	5´-AGGCCTGTCCACCATCAC-3´
AdipoR1 rev (m)	5´-CAGAAGGAGCCCCATTGC-3´
AdipoR1 uni (r)	5´-CGACAGGCCTAAGTGTCCAT-3´
AdipoR1 rev (r)	5´-CTTACCCTTCTCCTCCAGCA-3´
AdipoR2 uni (r)	5´-GAAGGAGGGTCAACTCACCA-3´
AdipoR2 rev (r)	5´-CATCAAGTTGGTGCCCTTTT-3´
β-actin uni (m)	5'-TGGAATCCTGTGGCATCCATG-3´
β-actin rev (m)	5'-TAAAACGCAGCTCAGTAACAG-3´
adiponectin (h)	5´-CATGACCAGGAAACCACGACT´-3´
adiponectin (h)	5´-TGAATGCTGAGCGGTAT-3´

h: Human; m: Mouse; r: Rat.

SDS-PAGE and immunoblotting

The cells or tissues were solubilized in RIPA buffer. Proteins were separated by SDS-polyacrylamide gel electrophoresis and were transferred to PVDF membranes (Bio-Rad, Germany). Incubations with antibodies were performed in 1% BSA in PBS, 0.1% Tween overnight. Detection of the immune complexes was carried out with the ECL Western blot detection system (Amersham Pharmacia, Deisenhofen, Germany).

Statistical analysis

Data are represented as Box Plots (Sigma Plot) indicating the median, the upper and lower quartile, the largest and the lowest value in the data set. Statistical differences were analysed by Student´s *t*-test (MS Excel) and a value of $P < 0.05$ was regarded as statistically significant.

RESULTS

Expression of AdipoR1 was analysed in HepG2 cells treated with insulin or leptin. HepG2 cells were treated

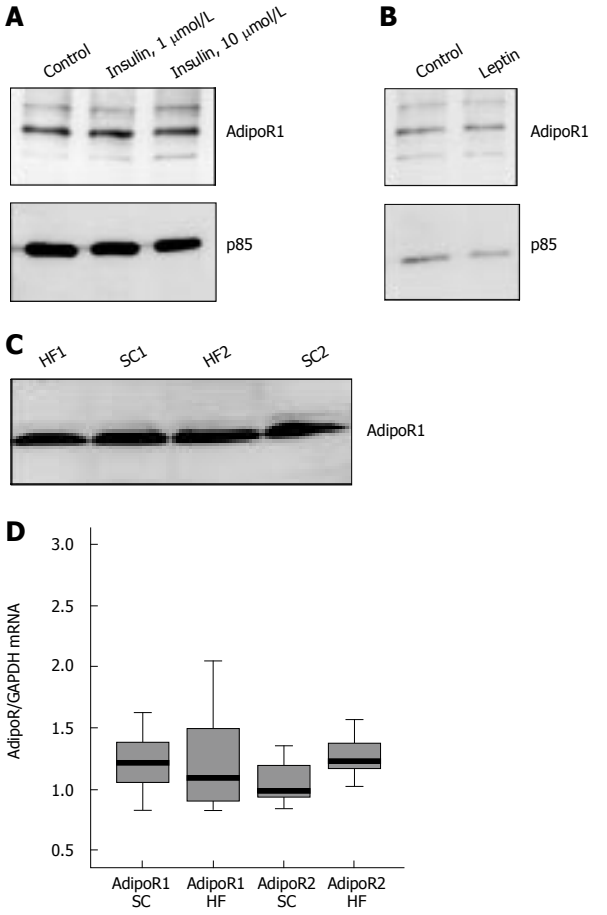


Figure 1 AdipoR1 mRNA and protein and AdipoR2 mRNA in fatty liver disease. **A**: Expression of AdipoR1 in HepG2 incubated with 1 μmol/L or 10 μmol/L insulin for 4 h; **B**: Analysis of AdipoR1 in HepG2 treated with recombinant leptin for 24 h; **C**: AdipoR1 abundance in the liver of rats kept on a standard chow (SC) or a high fat diet (HF); **D**: AdipoR1 and AdipoR2 mRNA expression in the liver of rats kept on a standard chow (SC) or a high fat diet (HF). For normalization GAPDH mRNA levels were determined.

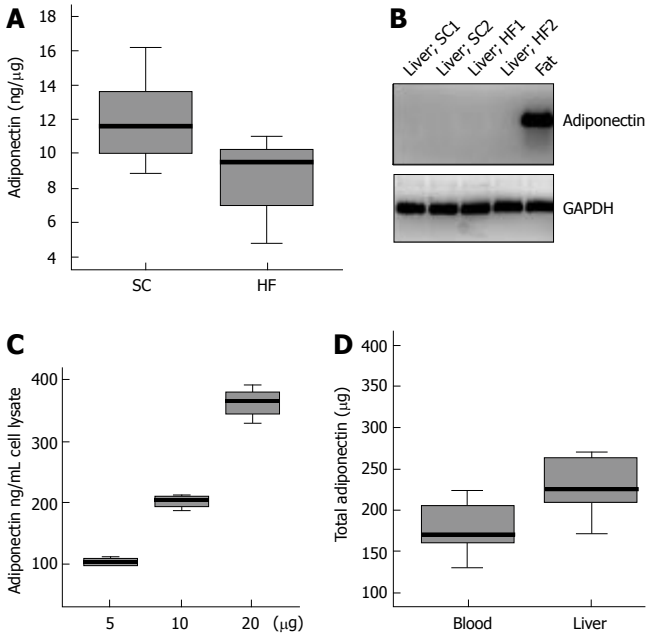


Figure 2 Hepatic adiponectin protein and mRNA in fatty liver disease. **A**: Adiponectin in the liver of standard chow (SC) and high fat diet (HF) animals. Concentration of adiponectin is ng per μg liver tissue; **B**: Adiponectin mRNA in the liver of rats kept on a standard chow (SC) or a high fat diet (HF). Fat tissue was analysed as a positive control; **C**: Primary hepatocytes were incubated with 5, 10 or 20 mg/L adiponectin for 24 h and adiponectin was determined in the cell lysates; **D**: Total systemic and hepatic adiponectin was calculated.

with insulin, 1 μmol/L and 10 μmol/L for four hours (Figure 1A) and six hours (not shown) or leptin for 24 h. AdipoR1 was analyzed by immunoblot and was found not to be regulated by insulin or leptin (Figures 1A and B). AdipoR1 and AdipoR2 mRNA expression was determined in insulin treated cells and was similar in controls and insulin-incubated HepG2 cells (not shown).

AdipoR1 protein and mRNA as well as AdipoR2 mRNA were analysed in the liver of rats on a standard chow (SC) or on a high fat (HF) diet. AdipoR1 protein level is similar in these animals (Figure 1C) and real-time RT-PCR revealed no alterations in the mRNA expression of AdipoR1 whereas AdipoR2 mRNA is induced with a relative expression of 1.0 ± 0.2 in SC and 1.3 ± 0.2 in the HF group (P = 0.004) (Figure 1D). RT-PCR data were normalized by the corresponding GAPDH values which were not different between the two groups.

Systemic and liver adiponectin were measured by ELISA. Circulating adiponectin is significantly reduced in fat-fed animals with 4.9 ± 1.1 mg/L adiponectin compared to 6.2 ± 8 mg/L in SC fed rats (P = 0.02) (not shown). Liver adiponectin is also lower in fat rats with 8.5 ± 2.4 ng adiponectin in 1 μg liver tissue when compared to controls with 11.9 ± 1.9 ng adiponectin (P = 0.01) (Figure 2A).

Adiponectin in the liver may be derived from the circulation or originate from liver cells. Adiponectin mRNA was analysed by RT-PCR with total RNA isolated from rat liver of HF and SC animals and rat adipose tissue as a positive control. Adiponectin mRNA was not detected in any RNA isolated from total liver by RT-PCR and 45 cycles of amplification but was easily amplified from adipose tissue RNA (Figure 2B). In addition, adiponectin mRNA was not detected in human liver, in isolated primary human hepatocytes or in HepG2 cells (not shown). Therefore hepatic adiponectin is most likely taken up by liver cells.

To test this hypothesis primary human hepatocytes were incubated with 5, 10 and 20 mg/L recombinant adiponectin for 24 h or PBS as solvent control in serum-free medium. Whereas adiponectin was not detected in the control cells and therefore is below 40 ng/L, adiponectin was found in the cell lysates of hepatocytes incubated with increasing amounts of recombinant protein and was 111 ± 3 μg/L, 200 ± 18 μg/L and 360 ± 43 μg/L, respectively (Figure 2C). This indicates that about 0.02% of the extracellular adiponectin is taken up by hepatocytes and the uptake correlates to the adiponectin concentration in the medium.

Total circulating adiponectin in rats was calculated and the blood volume was estimated as described (Blood volume (mL) = 0.06 × body weight + 0.77) [17]. Total liver adiponectin was also calculated by multiplication of hepatic adiponectin concentration with the corresponding weight of the liver. Adiponectin in the blood was 178 ± 31 μg and in the liver 241 ± 59 μg indicating that total liver adiponectin is higher than circulating adiponectin (P = 0.03) (Figure 2D). However, this is only a rough estimate because the blood volume of the liver was not included

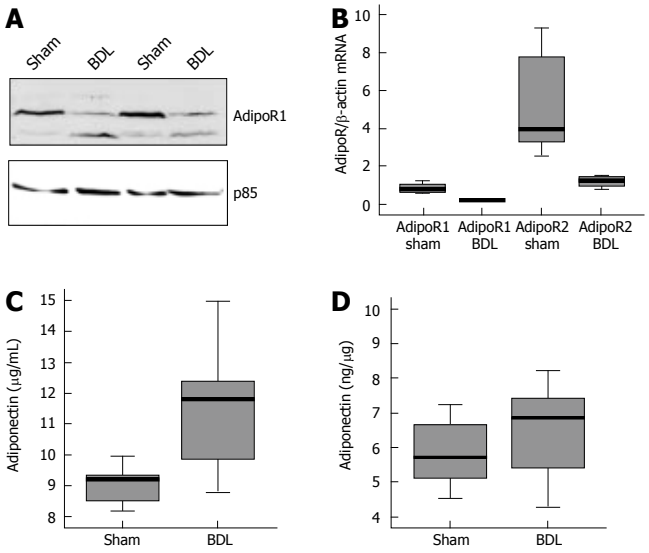


Figure 3 AdipoR1 and adiponectin in liver cirrhosis. **A**: AdipoR1 abundance in the liver of sham-operated or BDL mice; **B**: AdipoR1 and AdipoR2 mRNA expression in the liver of sham-operated or BDL mice. For normalization β-actin levels were determined; **C**: Systemic adiponectin in control and cholestatic animals; **D**: Adiponectin in the liver of sham-operated or BDL mice. Concentration of adiponectin is mg per g liver tissue.

in the calculation. Furthermore, adiponectin may be not equally distributed in the whole liver and levels may vary between different biopsies taken from the same organ.

AdipoR1 and AdipoR2 were also determined in a rodent model of liver cirrhosis. AdipoR1 protein was analysed in the liver of control mice and animals after BDL by immunoblot and was found reduced in all BDL animals investigated (Figure 3A). AdipoR1 and AdipoR2 mRNA were determined by real-time RT-PCR in control and BDL mice. Relative abundance of AdipoR1 mRNA was 0.8 ± 0.3 in sham and 0.2 ± 0.1 in BDL mice (P = 0.0004), AdipoR2 mRNA was 5.2 ± 2.6 in controls and 1.2 ± 0.3 in BDL animals (P = 0.007) (Figure 3B). Normalization was performed using β-actin as housekeeping gene which was similarly expressed in both groups (P = 0.14). Systemic adiponectin was significantly higher in the BDL group with 9.0 ± 0.6 mg/L in the control and 11.1 ± 2.0 mg/L in the BDL animals (P = 0.03) (Figure 3C) whereas hepatic adiponectin was similar with 5.8 ± 1.0 mg/g in sham and 6.4 ± 1.5 mg/g in the BDL mice (P = 0.4) (Figure 3D).

To identify mediators that are responsible for the suppression of AdipoR1 in liver cirrhosis, primary hepatocytes and HepG2 cells were incubated for 24 h with either LPS (1 and 10 mg/L), TNF (10 μg/L), supernatants of activated hepatic stellate cells, CCl_4 (1 mmol/L and 3 mmol/L) or actinomycin D (10 mg/L). AdipoR1 protein was not found reduced in cells treated with these mediators (not shown).

DISCUSSION

Although AdipoR2 has been suggested to represent the main adiponectin receptor in the liver we recently demonstrated significant expression of AdipoR1 mRNA and protein in primary human hepatocytes and HepG2 cells[8]. In the current study AdipoR1 mRNA and protein, AdipoR2 mRNA and abundance of circulating and hepatic adiponectin in rodent models of steatotic and cirrhotic liver was investigated.

Rats fed a HF diet develop insulin resistance and fatty liver disease[12]. AdipoR1 mRNA and protein is similar when total liver tissue isolated from rats on a standard diet or HF animals was analyzed. Furthermore, insulin did not alter AdipoR1 mRNA or protein in HepG2 cell. Leptin deficient ob/ob mice are obese and insulin resistant and hepatic AdipoR1 mRNA was found similarly expressed in these animals when compared to controls[18]. These data indicate that reduced insulin sensitivity is not associated with reduced AdipoR1 expression in the liver.

AdipoR2 mRNA is about 25% higher in the fatty liver of HF rats whereas in the ob/ob mice AdipoR2 mRNA is not altered[18]. AdipoR2 mRNA was also investigated in humans and is significantly elevated in fatty liver disease when compared to normal liver[19] in one study whereas a second study could not detect alterations in AdipoR2 mRNA[10,20]. However, protein expression has not been investigated so far but is important to clarify abundance of hepatic AdipoR2 in the metabolic syndrome.

Systemic adiponectin is significantly lower in the HF animals and reflects the human situation where circulating adiponectin has an inverse proportion to the body mass index[2]. In accordance with the lower systemic adiponectin levels, hepatic adiponectin is also reduced in the fat rat. Adiponectin mRNA was not detected in rat liver indicating that hepatic adiponectin may be taken up by the cells. Adiponectin was not found in primary human hepatocytes cultivated *in vitro* for three days but incubation of these cells with 5, 10 or 20 mg/L recombinant adiponectin for 24 h elevated cellular adiponectin in a concentration dependent way. This may indicate that adiponectin is taken up by hepatocytes either from the circulation or the portal vein.

The common and final state of all chronic liver diseases is liver cirrhosis. A well known model of extrahepatic biliary obstruction is common bile duct ligation in mice[21]. AdipoR1 mRNA and protein and AdipoR2 mRNA were found reduced in the cirrhotic liver of BDL animals. Systemic adiponectin is elevated in animals with liver cirrhosis, a phenomenon also described in a study investigating mice and humans[22]. However, hepatic adiponectin was similar in sham and BDL mice. It was suggested that adiponectin is excreted via the bile and an impaired liver function in cirrhosis may reduce biliary loss of circulating adiponectin explaining elevated systemic levels[22]. However, this hypothesis presumes reduced hepatic adiponectin concentrations when compared to control animals and therefore is not supported by our findings.

Although adiponectin was detected in the liver of mice and rats, adiponectin mRNA could not be amplified in rat liver, human liver or isolated human hepatocytes indicating that adiponectin is not produced by hepatocytes or other cells of the liver tissue. These results are in agreement with several studies[10,20] but contradict the results of other reports[11,22] and there is currently no explanation for these different findings. However, hepatocytes take up significant amounts of adiponectin and this may explain at least in

part why adiponectin is detected in the liver.

One limitation of the current study is that only four to eight animals could be analyzed per experiment and the investigations would benefit from a higher number of rodents. Nevertheless low circulating adiponectin in obesity[2] and high levels in liver cirrhosis[22] have also been reported in human studies and are in accordance with our findings.

All in all, our experiments show reduced circulating adiponectin in a rodent model of fatty liver disease and elevated adiponectin and diminished expression of adiponectin receptors in BDL-induced liver cirrhosis in mice.

ACKNOWLEDGMENTS

The expert technical assistance of Kerstin Winkler, Iris Ottinger and Anja Graebe is greatly appreciated.

REFERENCES

1 **McCullough AJ**. Update on nonalcoholic fatty liver disease. *J Clin Gastroenterol* 2002; **34**: 255-262
2 **Gil-Campos M**, Cañete RR, Gil A. Adiponectin, the missing link in insulin resistance and obesity. *Clin Nutr* 2004; **23**: 963-974
3 **Tsao TS**, Murrey HE, Hug C, Lee DH, Lodish HF. Oligomerization state-dependent activation of NF-kappa B signaling pathway by adipocyte complement-related protein of 30 kDa (Acrp30). *J Biol Chem* 2002; **277**: 29359-29362
4 **Hui JM**, Hodge A, Farrell GC, Kench JG, Kriketos A, George J. Beyond insulin resistance in NASH: TNF-alpha or adiponectin? *Hepatology* 2004; **40**: 46-54
5 **Xu A**, Wang Y, Keshaw H, Xu LY, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 2003; **112**: 91-100
6 **Czaja MJ**. Liver injury in the setting of steatosis: crosstalk between adipokine and cytokine. *Hepatology* 2004; **40**: 19-22
7 **Yamauchi T**, Kamon J, Ito Y, Tsuchida A, Yokomizo T, Kita S, Sugiyama T, Miyagishi M, Hara K, Tsunoda M, Murakami K, Ohteki T, Uchida S, Takekawa S, Waki H, Tsuno NH, Shibata Y, Terauchi Y, Froguel P, Tobe K, Koyasu S, Taira K, Kitamura T, Shimizu T, Nagai R, Kadowaki T. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; **423**: 762-769
8 **Neumeier M**, Weigert J, Schäffler A, Weiss T, Kirchner S, Laberer S, Schölmerich J, Buechler C. Regulation of adiponectin receptor 1 in human hepatocytes by agonists of nuclear receptors. *Biochem Biophys Res Commun* 2005; **334**: 924-929
9 **Wang Y**, Lam KS, Xu JY, Lu G, Xu LY, Cooper GJ, Xu A. Adiponectin inhibits cell proliferation by interacting with several growth factors in an oligomerization-dependent manner. *J Biol Chem* 2005; **280**: 18341-18347
10 **Vuppalanchi R**, Marri S, Kolwankar D, Considine RV, Chalasani N. Is adiponectin involved in the pathogenesis of nonalcoholic steatohepatitis? A preliminary human study. *J Clin Gastroenterol* 2005; **39**: 237-242
11 **Kaser S**, Moschen A, Cayon A, Kaser A, Crespo J, Pons-Romero F, Ebenbichler CF, Patsch JR, Tilg H. Adiponectin and its receptors in non-alcoholic steatohepatitis. *Gut* 2005; **54**: 117-121
12 **Buettner R**, Ottinger I, Schölmerich J, Bollheimer LC. Preserved direct hepatic insulin action in rats with diet-induced hepatic steatosis. *Am J Physiol Endocrinol Metab* 2004; **286**: E828-E833
13 **Hellerbrand SC,** Tsukamoto H, Brenner DA, Rippe RA. Expression of intracellular adhesion molecule 1 by activated hepatic stellate cells. *Hepatology* 1996; **24**: 670-676
14 **Weiss TS**, Pahernik S, Scheruebl I, Jauch KW, Thasler WE. Cellular damage to human hepatocytes through repeated application of 5-aminolevulinic acid. *J Hepatol* 2003; **38**: 476-482
15 **Thasler WE**, Weiss TS, Schillhorn K, Stoll PT, Irrgang B, Jauch KW. Charitable State-Controlled Foundation Human Tissue and Cell Research: Ethic and Legal Aspects in the Supply of Surgically Removed Human Tissue For Research in the Academic and Commercial Sector in Germany. *Cell Tissue Bank* 2003; **4**: 49-56
16 **Buechler C**, Ullrich H, Aslanidis C, Bared SM, Lingenhel A, Ritter M, Schmitz G. Lipoprotein (a) downregulates lysosomal acid lipase and induces interleukin-6 in human blood monocytes. *Biochim Biophys Acta* 2003; **1642**: 25-31
17 **Lee HB**, Blaufox MD. Blood volume in the rat. *J Nucl Med* 1985; **26**: 72-76
18 **Tsuchida A**, Yamauchi T, Ito Y, Hada Y, Maki T, Takekawa S, Kamon J, Kobayashi M, Suzuki R, Hara K, Kubota N, Terauchi Y, Froguel P, Nakae J, Kasuga M, Accili D, Tobe K, Ueki K, Nagai R, Kadowaki T. Insulin/Foxo1 pathway regulates expression levels of adiponectin receptors and adiponectin sensitivity. *J Biol Chem* 2004; **279**: 30817-30822
19 **Takamura T**, Shimizu A, Matsuzawa N, Honda M, Kaneko S. Adiponectin receptor II in non-alcoholic fatty liver disease. *Gut* 2005; e-letter to reference 11
20 **Oana F**, Takeda H, Matsuzawa A, Akahane S, Isaji M, Akahane M. Adiponectin receptor 2 expression in liver and insulin resistance in db/db mice given a beta3-adrenoceptor agonist. *Eur J Pharmacol* 2005; **518**: 71-76
21 **Rodríguez-Garay EA**. Cholestasis: human disease and experimental animal models. *Ann Hepatol* 2003; **2**: 150-158
22 **Tacke F**, Wüstefeld T, Horn R, Luedde T, Srinivas Rao A, Manns MP, Trautwein C, Brabant G. High adiponectin in chronic liver disease and cholestasis suggests biliary route of adiponectin excretion in vivo. *J Hepatol* 2005; **42**: 666-673

S- Editor Wang GP **L- Editor** Schreyer AG **E- Editor** Ma WH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5495-5500
World Journal of Gastroenterology ISSN 1007-9327

CLINICAL RESEARCH

C-reactive protein is a prognostic indicator in patients with perihilar cholangiocarcinoma

Thomas Gerhardt, Sabine Milz, Michael Schepke, Georg Feldmann, Martin Wolff, Tilman Sauerbruch, Franz Ludwig Dumoulin

Thomas Gerhardt, Sabine Milz, Michael Schepke, Georg Feldmann, Tilman Sauerbruch, Franz Ludwig Dumoulin, Department of Internal Medicine I, University Hospital Bonn, Germany
Martin Wolff, Department of Surgery, University Hospital Bonn, Germany
Correspondence to: Dr. Thomas Gerhardt, Department of Internal Medicine I, University Hospital, Sigmund-Freud-Str. 25, D-53105 Bonn, Germany. thomas.gerhardt@ukb.uni-bonn.de
Telephone: +49-228-28715507 Fax: +49-228-28714323
Received: 2006-04-14 Accepted: 2006-05-25

Abstract

AIM: To evaluate prognostic indicators for the outcome of patients with perihilar extrahepatic cholangiocarcinoma in an unselected cohort.

METHODS: We retrospectively analyzed 98 patients with perihilar cholangiocarcinoma. Twenty-three patients (23.5%) underwent tumor resection. Patients with non-resectable tumors underwent either transpapillary or percutaneous transhepatic biliary drainage. Additionally, 32 patients (32.7%) received photodynamic therapy (PDT) and 18 patients (18.4%) systemic chemotherapy. Predefined variables at the time of diagnosis and characteristics considering the mode of treatment were entered into a Cox's proportional hazards model. Included in the analysis were age, tumor stage following the modified Bismuth-Corlette classification, bilirubin, prothrombin time (PT), C-reactive protein (CRP), carbohydrate antigen 19-9 (CA19-9), history of weight loss, surgical resection, chemotherapy and PDT.

RESULTS: The Kaplan-Meier estimate of overall median survival was 10.5 (95%CI: 8.4-12.6) mo. In the univariate analysis, low Bismuth stage, low CRP and surgical resection correlated significantly with better survival. In the multivariate analysis, only CRP (P = 0.005) and surgical resection (P = 0.029) were found to be independently predictive of survival in the cohort. Receiver operating characteristic (ROC) analysis identified a CRP level of 11.75 mg/L as the value associated with the highest sensitivity and specificity predicting a survival > 5 mo. Applying Kaplan-Meier analysis, patients with a CRP < 12 mg/L at the time of diagnosis had a significantly longer median survival than patients with higher values (16.2 *vs* 7.6 mo; P = 0.009).

CONCLUSION: This retrospective analysis identified CRP level at the time of diagnosis as a novel indicator for the prognosis of patients with perihilar cholangiocarcinoma. It should be evaluated in future prospective trials on this entity.

Key words: Perihilar cholangiocarcinoma; Prognostic factors; C-reactive protein; Resection; Outcome

Gerhardt T, Milz S, Schepke M, Feldmann G, Wolff M, Sauerbruch T, Dumoulin FL. C-reactive protein is a prognostic indicator in patients with perihilar cholangiocarcinoma. *World J Gastroenterol* 2006; 12(34): 5495-5500

http://www.wjgnet.com/1007-9327/12/5495.asp

INTRODUCTION

Cholangiocarcinoma is a rare tumor[1]. The global incidence varies between 0.5 and 1.1 per 100 000[2]. High-risk groups have been defined. Thus, the life-time risk of intrahepatic and extrahepatic cholangiocarcinoma among patients with primary sclerosing cholangitis (PSC) ranges between 8%-20%[3]. Further risk factors for the occurrence of cholangiocarcinoma are infections with liver flukes[4], hepatolithiasis[5], choledochal cysts[6] or application of thorotrast[7]. While recent data show that incidence and mortality rates of intrahepatic cholangiocarcinoma are increasing in several areas in the world, the incidence and mortality rates of extrahepatic carcinoma are declining[8]. The 5-year survival of patients with extrahepatic cholangiocarcinoma is poor and was found to be less than 20% in a large population-based epidemiological study from the United States[1].

Perihilar cholangiocarcinoma is mostly diagnosed at an advanced stage. Therefore, more than two-thirds of patients are not suitable for surgery due to either expansion of the tumor or age and comorbidity[9]. Nevertheless, the prognosis of patients undergoing tumor resection has improved in recent years owing to advancements in surgical techniques resulting in a more aggressive resectional approach[10,11]. Furthermore, liver transplantation may be an option in highly selected patients after neo-adjuvant

radiochemotherapy and invasive staging[12,13].

Prognostic factors predicting the outcome of patients undergoing tumor resection have recently been extensively evaluated[10,14]. In contrast, less attention has been paid to overall outcome and possible prognostic indicators in unselected patients suffering from cholangiocarcinoma. Therefore, we performed a retrospective analysis of 98 consecutive patients with perihilar cholangiocarcinoma treated at a tertiary medical center within a period of 5 years in order to identify the most relevant predictors of outcome.

MATERIALS AND METHODS

Data acquisition

Using our hospital database, we identified the records of 98 consecutive unselected patients with extrahepatic perihilar cholangiocarcinoma type Bismuth I to IV admitted to our hospital between October 1997 and March 2003. Charts were reviewed retrospectively. Data for further analysis were available from all patients.

Diagnostic criteria

Cholangiography showed a perihilar stricture in all patients. Positive histology and/or cytology were present in 68 (69.4%) patients. In the remaining patients, diagnosis was made by the coexistence of a CA19-9 serum level greater than 250 IU/L and typical findings at cholangiography, ultrasound and CT scan.

We recorded patients' age, gender, clinical presentation, tumor stage following the modified Bismuth-Corlette classification[9], laboratory parameters at presentation (blood count, CRP, bilirubin, alkaline phosphatase, GPT, CA19-9), histology, cytology, type of medical treatment and outcome including date of death.

Statistical analysis

Numeric data were recorded as median and range or 95% confidence intervals (95% CI). To identify prognostic factors, we used the Cox's proportional hazards regression analysis. Survival analysis was performed using the Kaplan-Meier method and comparisons were made employing the log rank test. The Mann-Whitney rank sum test was used for inter-group comparisons. Statistical analysis was performed using the SPSS®- (SPSS Inc., Chicago, IL, USA) and the StatView 5.0®-Software (Version for Windows; SAS Institute Inc., Cary, NC, USA). A P value less than 0.05 was considered statistically significant.

RESULTS

Demographics and results of initial evaluation

We conducted this retrospective analysis on 98 consecutive patients (female/male: 48/50) with a median age of 69.5 (range: 35.8-89.9) years. Two patients of the cohort were known to suffer from PSC. Major clinical symptoms at admission were jaundice (73.5%), weight loss (43.9%) and pruritus (33.7%), whereas pain (22.5%), ascites (11.2%) and fever (9.2%) were present in less than one third of patients. The tumors were described as Bismuth types I (n = 12), II (n = 7), III (n = 30) and IV (n = 49), respectively. The laboratory findings at time of diagnosis are given in Table 1. CA19-9 levels did not correlate significantly to either serum bilirubin level (r = 0.068; P = 0.54) or Bismuth stage (r = 0.085; P = 0.44). Higher CRP-levels correlated significantly to leukocyte count (r = 0.569; P < 0.0001), but did not depend on bilirubin levels (r = 0.153; P = 0.16) and tumor extent according to the Bismuth-Corlette classification (r = 0.160; P = 0.15).

Table 1 Laboratory findings at time of diagnosis

Value (normal range)	Median	Range	
Total bilirubin (0.1-1.2 mg/L)	9.1	0.3	38.3
Alkaline phosphatase (50-175 U/L)	375	26	2572
ALT (GPT) (- 19 U/L)	61.5	8	464
Leucocytes (4.3-10.5 G/L)	11	3.6	95
CRP (- 3 mg/L)	8.4	0.06	207
CA19-9 (0.25-20 U/L)	232.9	0.25	24385
PT (70%-130%)	98.5	36	190

ALT: Alanin-Amino-Transferase; GPT: Glutamat-Pyruvat-Transaminase; CRP: C-reactive protein; CA 19-9: Carbohydrate-Antigen 19-9; PT: Prothrombin time.

Modality of treatment

Explorative laparotomy was performed in 43 patients (43.9%), and tumor resection could be performed in 23 (23.5%) patients of the cohort. Surgical therapy consisted of resection of the extrahepatic bile-ducts in 9 (39.1%) patients, partial duodenopancreatectomy with hilar resection in 2 (8.7%) patients, hilar resection with right hemihepatectomy in 5 (21.7%) patients, hilar resection with left hemihepatectomy in 4 (17.4%) patients, hilar resection with atypical liver resection in 2 (8.7%) patients and hepatectomy with consecutive liver transplantation in 1 (4.3%) patient. The resected patients were younger, had a lower Bismuth stage and had lower levels of serum bilirubin at diagnosis than the patients who did not undergo surgery, whereas CRP at diagnosis did not differ significantly between both groups (Table 2).

Patients with non-resectable tumors underwent either transpapillary or percutaneous transhepatic biliary drainage. Sixty-two (82.6%) of these patients received unilateral or bilateral plastic stents as biliary endoprosthesis, whereas in 34 (45.3%) patients, metal stents were placed during the course of the disease. In 30 (40%) patients, a percutaneous drainage had to be placed on at least one occasion during their clinical course.

Fifty-one patients received additional therapy. This therapy consisted of intraluminal photodynamic therapy using porfimer sodium (Photofrin™, Axcan, Canada) in 32 patients and systemic chemotherapy in 18 patients.

Survival analysis

At the end of observation, 85 of 98 (86.7%) patients had deceased with a median survival of 8.8 (0.8-55.1) mo. Sixteen patients were alive with a median follow-up of 12.3 (1.4-71.7) mo. The Kaplan-Meier estimated overall median survival was 10.5 (95% CI: 8.4-12.6) mo (Figure 1).

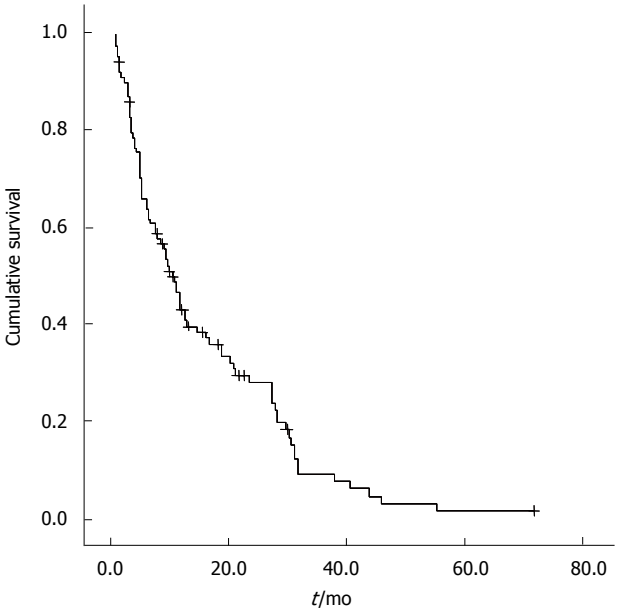


Figure 1 Kaplan-Meier estimate for survival of the whole cohort.

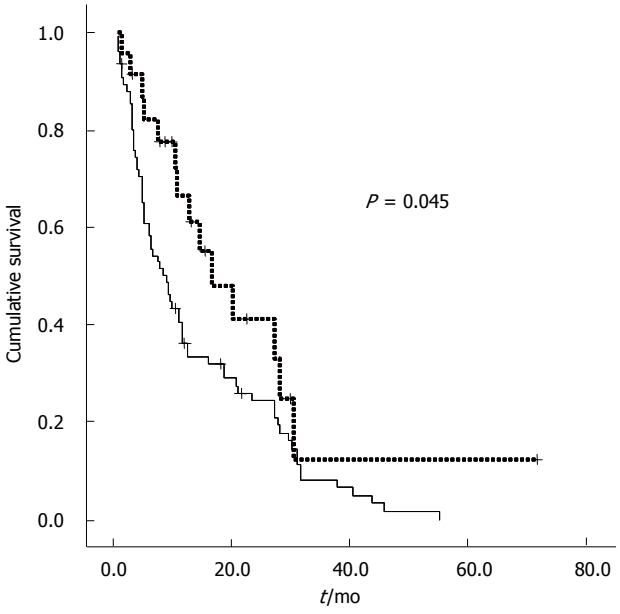


Figure 3 Kaplan-Meier estimate for survival of patients, who underwent resection (dotted line) *versus* non-resected patients (solid line).

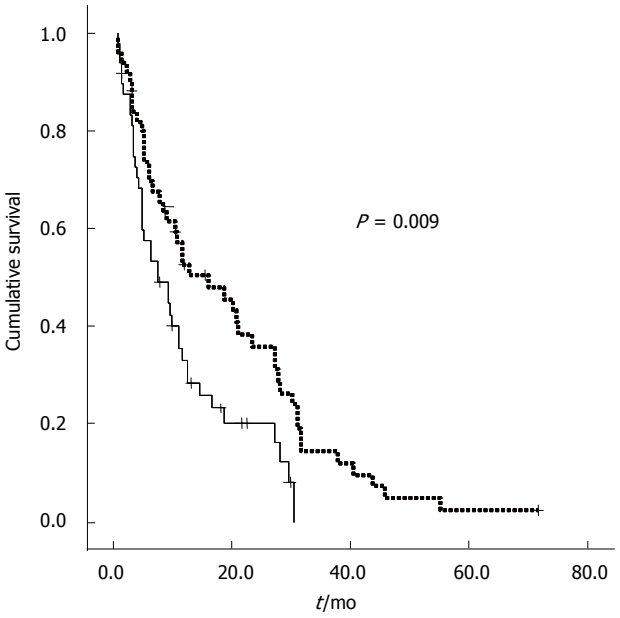


Figure 2 Kaplan-Meier estimate for patients with a serum CRP level < 12 mg/L (dotted line) *versus* ⩾ 12 mg/L (solid line).

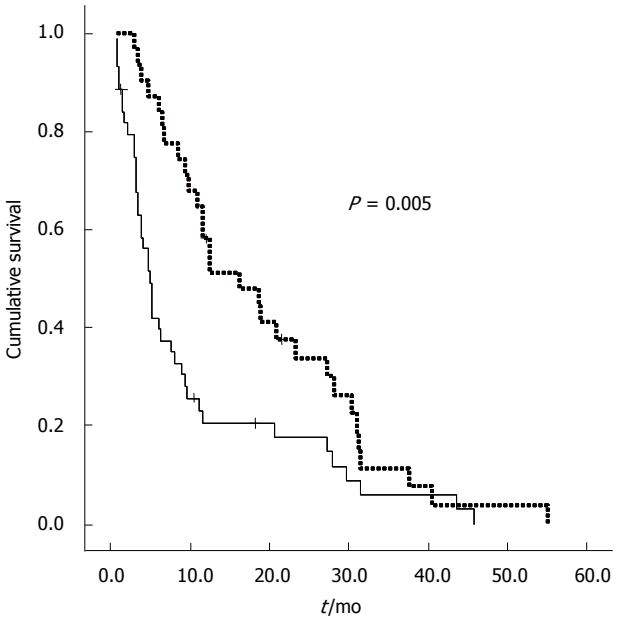


Figure 4 Subgroup analysis of patients without surgical resection: Kaplan-Meier estimate for survival of patients treated with PDT (dotted line) *versus* non-treated patients (solid line).

Prognostic factors and impact of treatment modality

The parameters examined and the results of the uni- and multivariate analyses are shown in Table 3. In the univariate analysis, low Bismuth stage, low CRP and surgical resection correlated significantly with better survival. In the multivariate analysis, only surgical resection ($P = 0.029$) and CRP ($P = 0.005$) were found to be independently predictive of survival in the cohort. ROC analysis identified a CRP level of 11.75 mg/L as the value associated with the highest sensitivity and specificity to identify patients surviving more than 5 mo.

Patients with a CRP level < 12 mg/L at the time of diagnosis had a significantly longer median estimated survival than patients with higher CRP values (16.2 *vs* 7.6 mo; $P = 0.009$) (Figure 2). The median survival in the subgroup of patients who underwent resection was significantly longer compared to patients receiving palliative treatment [16.6 (95% CI: 7.7-25.5) *vs* 9.0 (95% CI: 5.6-12.5); $P = 0.045$] (Figure 3). In contrast to the analysis of the whole cohort, in the subgroup of patients with irresectable tumors, PDT was associated with a significant improvement of survival [16.2 (95% CI: 7.0-25.5) *vs* 5.0 (95% CI: 3.8-6.3) mo ($P = 0.005$)] (Figure 4). Systemic chemotherapy was not correlated to a better outcome

Table 2 Inter-group comparison between patients undergoing surgical resection and patients with irresectable tumors

	Resection (n = 23)	No resection (n = 75)	P
Age	65.8 (36.4-74.1)	71.8 (35.8-90)	0.0003
Bismuth	Ⅱ (Ⅰ-Ⅳ)	Ⅳ (Ⅰ-Ⅳ)	0.001
Bilirubin	3.3 (0.3-17.5)	9.6 (0.4-38.3)	0.0026
CRP	15.6 (0.5-101)	9.9 (0.1-207)	0.445
CA19-9	71 (0.3-2910)	322 (0.3-24 385)	0.0098

Data are expressed as median (range).

Table 3 Results of univariate analysis for prognostic factors of survival

Variables	Hazard ratio	90% CI		P
Age	1.028	1.004	1.052	0.053
Bismuth type	0.784	0.656	0.927	0.023
Bilirubin	1.024	1.002	1.046	0.067
PT	0.996	0.989	1.004	0.402
CRP[a]	1.007	1.003	1.011	0.002
CA19-9	1.000	1.000	1.000	0.079
History of weight loss (yes/no)	1.125	0.783	1.615	0.592
Resection (yes/no)[a]	0.559	0.345	0.908	0.049
Chemotherapy (yes/no)	0.700	0.437	1.123	0.215
PDT (yes/no)	0.670	0.458	0.980	0.084

PT: Prothrombin time, CRP: C-reactive protein, CA 19-9: Carbohydrate-Antigen 19-9. Significant in the multivariate analysis ([a]P < 0.05).

neither in the multivariate analysis of the whole group nor in the subgroup of non-resected patients [11.6 (95% CI: 0.6-25) with chemotherapy *vs* 8.6 (95% CI: 5.0-12.2) mo without chemotherapy; P = 0.33].

DISCUSSION

Our study evaluated outcome and prognostic factors in a large series of unselected patients with perihilar cholangiocarcinoma treated at a tertiary medical center. The prognosis of these patients was poor. The median overall survival in our series was only 10.5 mo. Serum CRP level at diagnosis was identified as a new prognostic indicator for patients with perihilar cholangiocarcinoma. Surgical resection was also associated with prolonged survival. Moreover, in the subgroup of patients with irresectable tumors, additional therapy with PDT apart from biliary drainage, but not chemotherapy, was correlated with a better outcome. Certainly, particularly our data on the impact of treatment modalities on survival are influenced by all the restrictions of a retrospective analysis. There may be biases, such as selection for surgery and less complete follow-up in comparison to a prospective study. Unfortunately, prospective data on the clinical course of non-selected patients with perihilar cholangiocarcinoma are rare. Nevertheless, we were able to analyze a relatively large unselected cohort.

Prognostic factors in patients with cholangiocarcinoma undergoing resection have been extensively evaluated in retrospective series[11]. In a large series presented by Jarnagin *et al*[15], negative histologic margins, concomitant partial hepatectomy and a well-differentiated tumor were associated with an improved outcome. Accordingly, residual tumor as well as lymph node involvement were significant prognostic factors in a cohort of long-term-survivors[14]. Much less is known about the overall outcome of a more heterogeneous non-selected cohort with respect to its possible prognostic factors. Weight loss has previously been reported to be significantly associated with the outcome of patients with malignant strictures of the distal bile duct[16]. However, this factor could not be confirmed in our cohort of patients with perihilar tumors. Although a retrospective study of 49 cases of resected hilar cholangiocarcinoma identified total bilirubin greater than 10 mg/L to be associated with poorer survival[17], the bilirubin level was not significantly correlated to the outcome in our study. CRP, on the other hand, was a statistically significant prognostic factor, even in the multivariate analysis. Patients with a CRP < 12 mg/L at the time of diagnosis had a significantly longer median survival than patients with higher CRP values (16.2 *vs* 7.6 mo; P = 0.009). CRP belongs to the family of acute-phase proteins. Its concentration changes in response to injury, infection and neoplasia. It is up-regulated by cytokines, such as interleukin-8 (IL-8), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α)[18]. *In vitro* studies have identified IL-6 to be an autocrine growth factor of cholangiocarcinoma (CC) cell lines[19,20], in which it induces the expression of the anti-apoptotic protein Mcl-1[21]. Moreover, IL-6 was found to be markedly elevated in the serum of patients with CC and dropped sharply after resection[22]. Thus, high CRP levels might reflect an increased IL-6 level in patients with advanced cholangiocarcinoma. In this respect, the lack of IL-6 serum level determination displays a limitation of our study. In general, increased CRP levels in malignant disease could also be caused by an inflammatory response to tumor invasion[23]. Others showed in immunohistochemical studies that neoplastic tissue itself can express CRP[24]. In cholangiocarcinoma, one might also speculate that elevated CRP serum levels were caused by complicated tumor-induced strictures and subsequent cholangitis. Whereas in our study initial CRP levels correlated to leukocyte count, they were not significantly correlated to tumor size as assessed by the Bismuth-Corlette classification. Interestingly, increased serum CRP levels also correlated with shorter survival in patients with other gastrointestinal malignancies, including pancreatic, esophageal and colorectal cancer[25-27]. Recently, a CRP level ≤ 1.0 mg/dL was identified as favorable prognostic factor in a group of 65 patients with biliary tract cancers receiving chemotherapy[28]. However, this cohort consisted of 82% patients with gallbladder carcinoma, an entity with potentially different biological behavior and less frequent occurrence of cholestasis as compared to ours.

CA19-9 has been shown to be useful in the diagnostic evaluation of cholangiocarcinoma[29,30] and the resectability of intrahepatic and periampullary carcinomas[31,32]. Those of our patients who underwent resection had significantly lower CA19-9 levels at diagnosis, which might reflect a smaller tumor mass, but yet the marker was not correlated to overall outcome. This is in contrast to patients with

inoperable pancreatic cancer undergoing chemotherapy with gemcitabine, in whom CA 19-9 was prognostic[33].

The definitive role of chemotherapy and radiotherapy in the treatment of CC has not been fully established, although both options are commonly used[34]. In our cohort, a small number of patients receiving chemotherapy did not show favorable outcome compared to those without. Also PDT, which had been shown to be a promising palliative approach in several non-randomized and randomized studies on patients with irresectable cholangiocarcinoma, failed to be associated with favorable outcome in the overall analysis. However, it demonstrated a significant influence on survival in the subgroup of non-resected patients. Survival in these patients is comparable to previously published results from prospective trials[35-37].

In accordance with the literature, somewhat one fourth of our patients (24.8%) underwent surgical resection. In the univariate and the multivariate analyses, resection was significantly associated with a better outcome. Patients undergoing resection of their tumor were significantly younger, although age itself was not an independent prognostic parameter. Conclusions of the influence of tumor resection on the outcome of patients with perihilar CC in comparison to conservative treatment are clearly limited by the retrospective character of this analysis, which implements possible bias by patient selection.

In summary, our study evaluated the outcome of a heterogeneous non-selected cohort of patients with cholangiocarcinoma. In agreement with previous studies, surgical resection was identified as a prognostic factor for prolonged survival. In addition, the serum level of CRP at diagnosis was identified as a novel and independent prognostic indicator in patients suffering from perihilar cholangiocarcinoma and should, therefore, be considered as a prognostic parameter in the design of future prospective studies on this kind of patients.

REFERENCES

1 **Carriaga MT**, Henson DE. Liver, gallbladder, extrahepatic bile ducts, and pancreas. *Cancer* 1995; **75**: 171-190
2 **Strom BL**, Hibberd PL, Soper KA, Stolley PD, Nelson WL. International variations in epidemiology of cancers of the extrahepatic biliary tract. *Cancer Res* 1985; **45**: 5165-5168
3 **Kornfeld D**, Ekbom A, Ihre T. Survival and risk of cholangiocarcinoma in patients with primary sclerosing cholangitis. A population-based study. *Scand J Gastroenterol* 1997; **32**: 1042-1045
4 **Parkin DM**, Srivatanakul P, Khlat M, Chenvidhya D, Chotiwan P, Insiripong S, L'Abbé KA, Wild CP. Liver cancer in Thailand. I. A case-control study of cholangiocarcinoma. *Int J Cancer* 1991; **48**: 323-328
5 **Chen MF**. Peripheral cholangiocarcinoma (cholangiocellular carcinoma): clinical features, diagnosis and treatment. *J Gastroenterol Hepatol* 1999; **14**: 1144-1149
6 **Lipsett PA**, Pitt HA, Colombani PM, Boitnott JK, Cameron JL. Choledochal cyst disease. A changing pattern of presentation. *Ann Surg* 1994; **220**: 644-652
7 **Hardell L**, Bengtsson NO, Jonsson U, Eriksson S, Larsson LG. Aetiological aspects on primary liver cancer with special regard to alcohol, organic solvents and acute intermittent porphyria--an epidemiological investigation. *Br J Cancer* 1984; **50**: 389-397
8 **Shaib Y**, El-Serag HB. The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 115-125
9 **Bismuth H**, Nakache R, Diamond T. Management strategies in resection for hilar cholangiocarcinoma. *Ann Surg* 1992; **215**: 31-38
10 **Jarnagin WR**, Ruo L, Little SA, Klimstra D, D'Angelica M, DeMatteo RP, Wagman R, Blumgart LH, Fong Y. Patterns of initial disease recurrence after resection of gallbladder carcinoma and hilar cholangiocarcinoma: implications for adjuvant therapeutic strategies. *Cancer* 2003; **98**: 1689-1700
11 **Jarnagin WR**, Shoup M. Surgical management of cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 189-199
12 **Heimbach JK**, Gores GJ, Haddock MG, Alberts SR, Nyberg SL, Ishitani MB, Rosen CB. Liver transplantation for unresectable perihilar cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 201-207
13 **Heimbach JK**, Haddock MG, Alberts SR, Nyberg SL, Ishitani MB, Rosen CB, Gores GJ. Transplantation for hilar cholangiocarcinoma. *Liver Transpl* 2004; **10**: S65-S68
14 **Klempnauer J**, Ridder GJ, Werner M, Weimann A, Pichlmayr R. What constitutes long-term survival after surgery for hilar cholangiocarcinoma? *Cancer* 1997; **79**: 26-34
15 **Jarnagin WR**, Fong Y, DeMatteo RP, Gonen M, Burke EC, Bodniewicz BS J, Youssef BA M, Klimstra D, Blumgart LH. Staging, resectability, and outcome in 225 patients with hilar cholangiocarcinoma. *Ann Surg* 2001; **234**: 507-17; discussion 517-19
16 **Prat F**, Chapat O, Ducot B, Ponchon T, Fritsch J, Choury AD, Pelletier G, Buffet C. Predictive factors for survival of patients with inoperable malignant distal biliary strictures: a practical management guideline. *Gut* 1998; **42**: 76-80
17 **Su CH**, Tsay SH, Wu CC, Shyr YM, King KL, Lee CH, Lui WY, Liu TJ, P'eng FK. Factors influencing postoperative morbidity, mortality, and survival after resection for hilar cholangiocarcinoma. *Ann Surg* 1996; **223**: 384-394
18 **Castell** JV, Gómez-Lechón MJ, David M, Fabra R, Trullenque R, Heinrich PC. Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. *Hepatology* 1990; **12**: 1179-1186
19 **Okada K**, Shimizu Y, Nambu S, Higuchi K, Watanabe A. Interleukin-6 functions as an autocrine growth factor in a cholangiocarcinoma cell line. *J Gastroenterol Hepatol* 1994; **9**: 462-467
20 **Park J**, Tadlock L, Gores GJ, Patel T. Inhibition of interleukin 6-mediated mitogen-activated protein kinase activation attenuates growth of a cholangiocarcinoma cell line. *Hepatology* 1999; **30**: 1128-1133
21 **Meng F**, Yamagiwa Y, Ueno Y, Patel T. Over-expression of interleukin-6 enhances cell survival and transformed cell growth in human malignant cholangiocytes. *J Hepatol* 2006; **44**: 1055-1065
22 **Goydos JS**, Brumfield AM, Frezza E, Booth A, Lotze MT, Carty SE. Marked elevation of serum interleukin-6 in patients with cholangiocarcinoma: validation of utility as a clinical marker. *Ann Surg* 1998; **227**: 398-404
23 **Morley JJ**, Kushner I. Serum C-reactive protein levels in disease. *Ann N Y Acad Sci* 1982; **389**: 406-418
24 **Nozoe T**, Korenaga D, Futatsugi M, Saeki H, Maehara Y, Sugimachi K. Immunohistochemical expression of C-reactive protein in squamous cell carcinoma of the esophagus - significance as a tumor marker. *Cancer Lett* 2003; **192**: 89-95
25 **Falconer JS**, Fearon KC, Ross JA, Elton R, Wigmore SJ, Garden OJ, Carter DC. Acute-phase protein response and survival duration of patients with pancreatic cancer. *Cancer* 1995; **75**: 2077-2082
26 **Nozoe T**, Matsumata T, Kitamura M, Sugimachi K. Significance of preoperative elevation of serum C-reactive protein as an indicator for prognosis in colorectal cancer. *Am J Surg* 1998; **176**: 335-338
27 **Nozoe T**, Saeki H, Sugimachi K. Significance of preoperative elevation of serum C-reactive protein as an indicator of prognosis in esophageal carcinoma. *Am J Surg* 2001; **182**: 197-201
28 **Saisho T**, Okusaka T, Ueno H, Morizane C, Okada S. Prognostic factors in patients with advanced biliary tract cancer receiving chemotherapy. *Hepatogastroenterology* 2005; **52**: 1654-1658

29 **Patel AH**, Harnois DM, Klee GG, LaRusso NF, Gores GJ. The utility of CA 19-9 in the diagnoses of cholangiocarcinoma in patients without primary sclerosing cholangitis. *Am J Gastroenterol* 2000; **95**: 204-207
30 **Qin XL**, Wang ZR, Shi JS, Lu M, Wang L, He QR. Utility of serum CA19-9 in diagnosis of cholangiocarcinoma: in comparison with CEA. *World J Gastroenterol* 2004; **10**: 427-432
31 **Ohtsuka M**, Ito H, Kimura F, Shimizu H, Togawa A, Yoshidome H, Miyazaki M. Results of surgical treatment for intrahepatic cholangiocarcinoma and clinicopathological factors influencing survival. *Br J Surg* 2002; **89**: 1525-1531
32 **Kau SY**, Shyr YM, Su CH, Wu CW, Lui WY. Diagnostic and prognostic values of CA 19-9 and CEA in periampullary cancers. *J Am Coll Surg* 1999; **188**: 415-420
33 **Ziske C**, Schlie C, Gorschlüter M, Glasmacher A, Mey U, Strehl J, Sauerbruch T, Schmidt-Wolf IG. Prognostic value of CA 19-9 levels in patients with inoperable adenocarcinoma of the pancreas treated with gemcitabine. *Br J Cancer* 2003; **89**: 1413-1417
34 **Thongprasert S**. The role of chemotherapy in cholangiocarcinoma. *Ann Oncol* 2005; **16** Suppl 2: ii93-ii96
35 **Dumoulin FL**, Gerhardt T, Fuchs S, Scheurlen C, Neubrand M, Layer G, Sauerbruch T. Phase II study of photodynamic therapy and metal stent as palliative treatment for nonresectable hilar cholangiocarcinoma. *Gastrointest Endosc* 2003; **57**: 860-867
36 **Berr F**, Wiedmann M, Tannapfel A, Halm U, Kohlhaw KR, Schmidt F, Wittekind C, Hauss J, Mössner J. Photodynamic therapy for advanced bile duct cancer: evidence for improved palliation and extended survival. *Hepatology* 2000; **31**: 291-298
37 **Ortner ME**, Caca K, Berr F, Liebetruth J, Mansmann U, Huster D, Voderholzer W, Schachschal G, Mössner J, Lochs H. Successful photodynamic therapy for nonresectable cholangiocarcinoma: a randomized prospective study. *Gastroenterology* 2003; **125**: 1355-1363

S- Editor Liu Y **L- Editor** Kumar M **E- Editor** Bai SH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5501-5508
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Intensity-modulated radiation therapy with concurrent chemotherapy for locally advanced cervical and upper thoracic esophageal cancer

Shu-Lian Wang, Zhongxing Liao, Helen Liu, Jaffer Ajani, Stephen Swisher, James D Cox, Ritsuko Komaki

Shu-Lian Wang, Department of Radiation Oncology, Cancer Hospital, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China
Zhongxing Liao, Helen Liu, James D Cox, Ritsuko Komaki, Department of Radiation Oncology, the University of Texas M D Anderson Cancer Center, United States
Jaffer Ajani, Department of Gastrointestinal Medical Oncology, the University of Texas M D Anderson Cancer Center, United States
Stephen Swisher, Department of Cardiovascular and Thoracic Surgical Oncology, the University of Texas M D Anderson Cancer Center, United States
Supported by Radiology Society of Northern America Researh and Education Program, Grant to "Teach the Teachers" from Emerging Nations
Correspondence to: Zhongxing Liao, MD, Department of Radiation Oncology, the University of Texas M D Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 97, Houston, TX 77030, United States. zliao@mdanderson.org
Telephone: +1-713-56302300 Fax: +1-713-5632366
Received: 2005-12-14 Accepted: 2006-05-23

Abstract

AIM: To evaluate the dosimetry, efficacy and toxicity of intensity-modulated radiation therapy (IMRT) and concurrent chemotherapy for patients with locally advanced cervical and upper thoracic esophageal cancer.

METHODS: A retrospective study was performed on 7 patients who were definitively treated with IMRT and concurrent chemotherapy. Patients who did not receive IMRT radiation and concurrent chemotherapy were not included in this analysis. IMRT plans were evaluated to assess the tumor coverage and normal tissue avoidance. Treatment response was evaluated and toxicities were assessed.

RESULTS: Five- to nine-beam IMRT were used to deliver a total dose of 59.4-66 Gy (median: 64.8 Gy) to the primary tumor with 6-MV photons. The minimum dose received by the planning tumor volume (PTV) of the gross tumor volume boost was 91.2%-98.2% of the prescription dose (standard deviation [SD]: 3.7%-5.7%). The minimum dose received by the PTV of the clinical tumor volume was 93.8%-104.8% (SD: 4.3%-11.1%) of the prescribed dose. With a median follow-up of 15 mo (range: 3-21 mo), all 6 evaluable patients achieved complete response. Of them, 2 developed local recurrences and 2 had distant metastases, 3 survived with no evidence of disease. After treatment, 2 patients developed esophageal stricture requiring frequent dilation and 1 patient developed tracheal-esophageal fistula.

CONCLUSION: Concurrent IMRT and chemotherapy resulted in an excellent early response in patients with locally advanced cervical and upper thoracic esophageal cancer. However, local and distant recurrence and toxicity remain to be a problem. Innovative approaches are needed to improve the outcome.

Key words: Esophageal cancer; Intensity-modulated radiation therapy; Chemotherapy

Wang SL, Liao Z, Liu H, Ajani J, Swisher S, Cox JD, Komaki R. Intensity-modulated radiation therapy with concurrent chemotherapy for locally advanced cervical and upper thoracic esophageal cancer. *World J Gastroenterol* 2006; 12(34): 5501-5508

http://www.wjgnet.com/1007-9327/12/5501.asp

INTRODUCTION

Cervical esophageal cancer occurs rarely and accounts for only 2%-10% of all esophageal carcinomas in the United States[1]. Surgery, an option only for patients with early-stage tumors, generally requires a total laryngopha ryngoesophagectomy with reconstruction, an operation often leads to considerable dysfunction. The 5-year overall survival rate was only 16%-28% in two studies of patients treated with curative surgery[2,3]. Unfortunately, surgical resection is not an appropriate treatment for those with locally advanced tumors, because it is difficult to achieve a clear margin. Radiotherapy alone, which does preserve laryngeal function, produces poor results, as evidenced by two studies of patients treated with definitive radiotherapy alone, showing the 5-year overall survival rate of only 14%-25%[4,5].

Concurrent chemoradiotherapy is now standard treatment for locally advanced esophageal cancer, based on the results of an intergroup randomized controlled trial

(RTOG 8501)[6]. Four studies examined the results from RTOG trial among small cohorts of cervical esophageal cancer patients who were treated with concurrent chemoradiotherapy[7-10]. The 5-year survival rate was 55% in one[9], and the 10-year survival rate was 27% in another[8].

As to radiotherapy, treatment planning for cervical esophageal cancer is challenging partly because of the anatomical structures involved. The cervical esophagus lies in a close proximity to the spinal cord, courses through the lower neck and upper thorax with drastic change of contour and diameter of the anatomy; and the lymph nodes at risk must be incorporated into the irregular treatment volume.

Evidence suggested that, in these patients, high-dose radiotherapy results in better local control and survival compared with low-dose radiotherapy[11] However, high doses of radiotherapy may be associated with potentially high risk of complication because of the adjacent critical structure involved in the high radiation region, such as spinal cord.

Intensity-modulated radiation therapy (IMRT) is a novel approach to the planning and delivery of radiation therapy. Numerous investigators have demonstrated the benefits of IMRT planning in a variety of tumor sites in terms of the feasibility of normal tissue sparing[12-14] and the delivery of higher radiation doses than conventional doses[15,16]. Another advantage of IMRT is its ability to deliver differentiated dose to various structure during same fraction dose irradiation, thus allows to deliver a higher dose to gross tumor and lower dose to subclinical disease during same session of beam deliver, as commonly described as "simultaneous integrated boost (SIB)".

In two recent studies of esophageal cancer, IMRT plans were found to be superior to three-dimensional conformal radiation therapy (3D-CRT) plans in terms of dose conformity, homogeneity, and sparing of critical normal structure, such as the spinal cord and lung[17,18]. An another study demonstrated that IMRT plans produced better dosimetric results than 3D-CRT in patients with cervical esophageal cancer[19]. However, there is no clinical treatment result reported on patients with cervical esophageal cancer who were treated with IMRT.

At the University of Texas MD Anderson Cancer Center, we have used IMRT concurrently with chemotherapy for all patients with cervical esophageal cancer and for some with upper thoracic esophageal cancer since August 2002. We performed a retrospective study to evaluate the dosimetric considerations, early response and toxicity of this group of patients.

MATERIALS AND METHODS

Patient population

In this retrospective study, conducted from August 1, 2002 through December 31, 2004, 7 patients were selected according to the following inclusion criteria: newly diagnosed locally advanced cervical and upper thoracic esophageal cancer, definitive concurrent IMRT with chemotherapy treatment at the University of Texas MD Anderson Cancer Center, recoverable treatment plan available. Patients were excluded if they had a distant metastasis or had esophagectomy or previously had radiotherapy in the neck or thoracic region. This study was approved by our Institutional Review Board, and informed consent for radiation therapy from all patients was taken. HIPPA compliance was enforced.

Pretreatment evaluation

For their pretreatment evaluation, all patients underwent computed tomography (CT) of the neck, chest, and abdomen, positron emission tomography (PET)-CT, and endoscopy and biopsy of the esophagus. Five patients underwent endoscopic sonography of the esophagus; the other 2 patients did not undergo endoscopic ultrasound examination due to severe obstruction of the esophagus by tumor; instead, they underwent bronchoscopy and PET-CT, which confirmed that they had T4N0 and T4N1 disease. In total, 6 of the 7 patients underwent bronchoscopy. The 1997 American Joint Committee on Cancer guidelines for the staging of cancer were used to classify tumor stage. Tumor stage was determined by endoscopic sonography and bronchoscopy findings, nodal stage was determined by endoscopic sonography or PET-CT, and metastasis stage was determined by PET-CT.

Chemotherapy

The induction chemotherapy consisted of two cycles of weekly carboplatin and paclitaxel (n = 1), given before chemoradiation. The concurrent chemotherapy consisted of continuous infusion of 5-fluorouracil (5-FU) (1000 mg/m^2) on d 1 to 4 and 29 to 32 and cisplatin (75 mg/m^2) on d 1 and 29 (n = 2), continuous infusion of 5-FU (700 mg/m^2) on d 1 to 5 and 29 to 33 and cisplatin (75 mg/m^2) on d 1 and 29 (n = 1), continuous infusion of 5-FU (300 mg/m^2) on Monday to Friday for 5 wk and paclitaxel (45-50 mg/m^2) weekly (n = 2), carboplatin area under the curve (AUC) 2 twice weekly and paclitaxel (30 mg/m^2) weekly for 5 wk (n = 1), or carboplatin AUC 1 once weekly and docetaxel (20 mg/m^2) weekly and continuous infusion of 5-FU (200 mg/m^2) on Monday to Friday for 5 wk (n = 1).

Radiotherapy

All patients underwent CT simulation in a supine position with their arms by their sides; the CT images were taken at a 3-mm thickness throughout the entire neck and thorax. Four of the patients were immobilized with a head and neck/upper thoracic thermoplastic mask, and three with a vacuum-locked cradle. The gross tumor volume (GTV), clinical target volume (CTV), planning target volume (PTV), spinal cord, and lung parenchyma were outlined on each image. The GTV was defined as any visible tumor on the image. The CTV was defined as the GTV plus a 2- to 5-cm margin superior to the highest extension of the tumor and a 4- to 5-cm margin inferior to the lowest extension of the tumor with a 2-cm radial margin. Uninvolved bony structure and lung tissue were kept outside the CTV. The PTV was defined as the CTV plus a 5-mm margin. For patients in whom the SIB was used, the GTV boost was defined as the initial GTV plus a 1.5-cm surrounding margin. A 5-mm margin around normal structures, such as

the spinal cord and lung, was also added for the planning organ-at-risk volume (PRV).

The inverse IMRT plans for 4 of the 7 patients were created using Corvus software (v4.0) (Corvus, Nomos Inc., Sewickley, PA) and for the other 3 using Pinnacle (v6.2) software (Philips Radiation Oncology Systems, Andover, MA). All treatment plans used heterogeneity correction and were delivered with 6-MV photons. The mean dose, dose range, and standard deviation (SD) of the PTV (for GTV boost and CTV) were calculated. The minimum dose to the PTV was defined as the dose to the coldest 1% of target volume, and the maximum dose was defined as the dose to the hottest 1% of target volume. The SD percentage of the PTV was the SD dose to the PTV divided by the prescription dose to the PTV. The maximum dose to the spinal cord was defined as that received by 1 cm^3 of the volume. The mean dose and the lung V_{20} (volume of lung receiving $\geqslant$ 20 Gy) for the total lung were calculated. The lung $V_{20} < 40\%$ and the maximum spinal cord dose of 45 Gy were considered to be acceptable for the IMRT plan. The pretreatment dosimetric quality assurance procedure and a test run were performed before the start of radiotherapy. Radiotherapy was delivered in a step-and-shoot mode with multi-leaf collimators. Portal films were obtained weekly to ensure the correct set-up.

Toxicity assessment

Acute toxicity levels were assessed weekly with complete blood cell counts and examinations for esophagitis and skin reactions during the concurrent chemoradiotherapy. Esophageal toxicity was defined as late if it occurred more than 90 d after radiotherapy. Radiation pneumonitis was defined as acute if it occurred within 6 mo after radiotherapy. All toxic effects were assessed using RTOG criteria[20].

Follow-up evaluation

The following evaluations were performed 1 mo after treatment, every 3 mo for the first 2 years, and every 6 mo thereafter: physical examination, complete blood cell count, blood chemistry tests, an endoscopic examination, an esophageal biopsy, and scans of the neck, chest, and abdomen by CT, PET-CT, or both. Endoscopic sonography of the esophagus was performed in two of the patients to evaluate lymph node response.

Response assessment

A complete response (CR) of the primary tumor was defined as: (1) endoscopic examination was negative for all visible tumors and biopsy was negative for tumor cells lasted for $\geqslant$ 4 wk, and (2) no evidence of abnormal hypermetabolism on PET-CT scan. The response of the metastatic lymph nodes was assessed by CT, PET-CT, or endoscopic sonography of the esophagus. A CR was defined as the complete disappearance of all measurable and assessable disease for $\geqslant$ 4 wk.

Statistical analysis

The survival was calculated from the date of diagnosis.

RESULTS

Patients' characteristics

The median time of follow-up was 15 mo (range: 3-21 mo). Of the 7 patients included in this study, 6 were men. The Karnofsky performance status scores ranged from 70 to 90, and their ages ranged from 52 to 78 years. Six patients had cervical esophageal squamous cell carcinoma, and 1 patient had upper thoracic esophageal adenocarcinoma. At the time of presentation, 2 patients tolerated solid foods, 2 tolerated soft foods, and 3 tolerated only liquid diet. Three patients had lost more than 10% of their weight in the 3 mo before treatment.

Patients had locally advanced disease as follows: 3 patients had biopsy-proven tracheal invasion by tumor through bronchoscopy examination, rendered them as T_4 stage; one of them had 60% tracheal obstruction with a tracheal-esophageal (TE) fistula and had a tracheal stent placement before chemoradiation treatment; 3 patients had T3 and 1 had T2N1 stage tumors. According to the endoscopic and PET-CT findings, the tumor length ranged from 3 to 8 cm. In 6 patients, a percutaneous gastrostomy or jejunostomy feeding tube was placed prior to the start of radiotherapy because of severe dysphagia, poor nutritional status, or a high risk of aspiration. The characteristics of the patients, tumor, and treatment are summarized in Table 1.

Dosimetric considerations

The primary tumor was irradiated with a total dose of 59.4-66 Gy given in 1.8-2.31 Gy/fraction. Three patients with cervical esophageal cancer also received prophylactic irradiation to the bilateral supraclavicular regions to a total dose of 46-60 Gy at 1.36-1.8 Gy/fraction. One patient with cervical esophageal cancer received bilateral supraclavicular and neck irradiation to a total dose of 56 Gy at 1.7 Gy/fraction because of small lymph node metastasis in the bilateral lower neck diagnosed by PET-CT. Three patients with cervical esophageal cancer also received irradiation to the superior mediastinum to a total dose of 56-66 Gy at 1.7-2.0 Gy/fraction. The SIB technique was used in 5 of the 6 patients with cervical esophageal cancer. In the other 2 patients, the GTV and CTV received the same fraction dose and total dose, since lymph node region was not prophylactically treated. Table 1 also shows the radiation treatment characteristics. Five, seven, eight, or nine coplanar beams with different gantry angles were used in the 7 patients. The total number of beam segments and monitor units (MU) was 218-559 and 21 252-40 812 for Corvus plans, and 198-417 and 27 423-42 504 for Pinnacle plans, respectively (Table 2). The daily treatment time was about 10-20 min.

Table 3 summarizes the dosimetric parameters of IMRT plans for the 7 patients. The median GTV was 78 cm^3 (range: 17-229 cm^3). The median GTV dose was 64.8 Gy (range: 59.4-66 Gy), and the median CTV dose was 56 Gy (range: 50.4-66 Gy). The minimum dose received by the PTV of boost GTV ranged from 91.2 to 98.2% (SD: 3.7%-5.7%) of the prescribed dose. The minimum dose received by the PTV of CTV ranged from 93.8% to 104.8% (SD: 4.3%-11.1%) of the prescribed dose. The V_{20}

Table 1 Patient and treatment characteristics for the 7 patients with cervical and upper thoracic esophageal cancer

Patient No.	Tumor distance from UES (cm)	Stage	Induction chemotherapy	Concurrent chemotherapy	XRT dose (GTV)	XRT dose (CTV)	XRT dose (supraclavical regions)
1	2	$T_3N_1M_0$		5-FU, CDDP	64.8 Gy (2.31 Gy/f)	50.4 Gy (1.8 Gy/f)	50.4 Gy (1.8 Gy/f)
2	0	$T_2N_1M_0$		5-FU, paclitaxel	59.9 Gy, (2.14 Gy/f)	50.4 Gy (1.8 Gy/f)	0
3	0	$T_3N_1M_0$		5-FU, CDDP	64.8 Gy (1.8 Gy/f × 8, 2.21 Gy × 21)	51.7 Gy (1.53 Gy/f × 8, 1.88 Gy × 21)	46 Gy (1.36 Gy/f × 8, 1.67 Gy × 21)
4	6	$T_3N_0M_0$		5-FU, paclitaxel	59.4 Gy (1.8 Gy/f)	59.4 Gy (1.8 Gy/f)	0
5	0	$T_4N_1M_0$		5-FU, CBP, docetaxel	66 Gy (2.0 Gy/f)	66 Gy (2.0 Gy/f)	0
6	2.5	$T_4N_1M_0$	CBP, paclitaxel	CBP, paclitaxel	66 Gy (2.0 Gy/f)	56 Gy (1.7 Gy/f)	56 Gy (1.7 Gy/f)
7	0	$T_4N_0M_0$		5-FU, CDDP	66 Gy (2.0 Gy/f)	60 Gy (1.8 Gy/f)	60 Gy (1.8 Gy/f)

CBP: Carboplatin; CDDP: Cisplatin; CTV: Clinical tumor volume; GTV: Gross tumor volume; f: Fraction; UES: Upper esophageal sphincter; XRT: Radiotherapy

to the bilateral lung was 10.3%-36.0% in all patients, and the lung mean dose was 6.6-17.6 Gy (median: 12.6 Gy). The maximum dose to the spinal cord ranged from 37.2 to 45.8 Gy. The dose distribution and dose volume histogram (DVH) for patient number 7 are shown in Figures 1 and 2 using pinnacle planning system.

Tumor response and failure

One patient with cervical esophageal cancer, whose general condition gradually deteriorated, experienced excessive mucus production, weight loss and fever, and decided to begin home hospice care after 2 wk of radiotherapy, died of disease 4 mo after diagnosis. Thus, this patient' s response was not evaluable and was not included in this analysis.

The other 6 patients all achieved a CR in the primary tumor and lymph node areas after concurrent chemoradiotherapy. The response was assessed by endoscopic biopsy for all patients, 4 of them also had PET-CT. Two patients had no evidence of disease (NED) at 13 and 17 mo. Two patients developed local recurrence in the esophagus 4 and 6 mo after treatment, one of them was successfully treated with salvage photodynamic therapy and had NED at 21 mo and the other died of disease at 15 mo. One patient had lung metastasis 7 mo after treatment and was alive with disease at 17 mo. One patient had both lymph nodes recurrence in the neck and soft tissue metastasis in the left thigh 11 mo after treatment and died of disease. Table 4 shows the tumor response and failure.

Toxicity

Acute major toxic effects included myelosuppression, dermatitis, and esophagitis. Myelosuppression occurred in 2 patients: 1 had grade 3 and another had grade 4 leukopenia. Three of the patients who had been immobilized with a thermoplastic mask experienced grade 3 skin reactions in the neck, and 1 of these required a 3-d treatment interruption. The patient who had a T4 tumor with a tracheoesophageal (TE) fistula and tracheal stent placement before chemoradiation developed grade 4 esophagitis 1 mo after treatment and had an esophageal stent placed 4 mo after treatment. Another patient experienced grade 4 late esophageal toxicity (TE fistula) 7 mo after radiotherapy and had an esophageal stent placed. Two patients experienced grade 3 late esophageal toxicity (benign esophageal strictures requiring esophageal dilatation one to four times) 4 mo and 10 mo after radiotherapy. No patient had symptomatic radiation pneumonitis, although 2 patients had radiographic changes in their irradiated lung. No patients lost more than 10% of their weight during chemoradiotherapy. No patient experienced radiation myelitis. All except 1 patient completed radiotherapy without interruption in 37-44 d. Radiotherapy was interrupted for three day in 1 patient due to grade 3 skin toxicity and radiation was completed in 50 d. Table 4 summarizes the toxicity of chemoradiotherapy.

Table 2 Beam arrangement of IMRT plans for the 7 patients with cervical and upper thoracic esophageal cancer

Number of beams	Gantry angles	Number of segments	Number of monitor units per fraction	Total fraction	Total monitor units
7	0, 25, 65, 141, 212, 295, 335	315	1395	28	39 060
5	40, 70, 220, 240, 290	218	759	28	21 252
9	0, 18, 35, 70, 150, 225, 295, 320, 340	559	1248 and 1468	8 + 21	40 812
7	0, 30, 60, 105, 260, 300, 330	270	749	33	24 717
5	0, 50, 120, 240, 300	198	831	33	27 423
8	12, 36, 60, 135, 225, 300, 324, 348	332	1288	33	42 504
9	0, 40, 70, 130, 160, 200, 230, 290, 320	417	972	33	32 076

DISCUSSION

No consensus has been reached as to the optimal radiation technique and target volume delineation for treating cervical esophageal cancer, and a survey conducted in Canada has come up with different opinions from radiation oncologists[21,22]. IMRT has potential benefit for treating cervical esophageal cancer because of the complexity of anatomy in this region. Separate IMRT plans designed for the initial large-field treatment and the subsequent boost

Table 3 Dosimetric results of IMRT for 7 patients with cervical and upper thoracic esophageal cancer

Patient No.	Dose (Gy)	GTV volume (cm^3)	PTV mean dose (Gy)	PTV min %	PTV max %	PTV SD %	Lung V_{20} (Gy)	Lung mean dose (Gy)	Spinal cord max (Gy)
1	64.8 (GTV)	58	70.5	96.5	117.9	4.9	18.9	12.0	42.8
	50.4 (CTV)		66.4	104.8	149.6	11.1			
	50.4 (sup)		61.3	101.6	149.2	11.9			
2	59.9 (GTV)	53	65.0	91.2	122.9	6.0	28.6	13.4	25.7
	50.4 (CTV)		58.7	103.9	139.4	6.6			
3	64.8 (GTV)	85	68.6	97.8	117.5	3.7	10.3	6.6	37.2
	51.7 (CTV)		62.3	93.8	121.2	9.2			
	46 (sup)		54.2	100	143	8.1			
4	59.4 (GTV)	17	65.0	97.1	119.1	4.3	24.2	11.6	39.2
5	66 (GTV)	78	67.3	97.9	109.2	4.9	26.8	12.6	45.8
6	66 (GTV)	229	68.0	98.2	110.9	4.8	21.9	7.7	40.2
	56 (CTV + neck)		59.8	101.1	122.9	9.4			
7	66 (GTC)	169	66.4	99.4	111.8	5.7	36	17.6	41.8
	60 (CTV)		65.7	97	121.6	6.7			
	60 (sup)		67.1	105	116.8	5.4			

GTV: Gross tumor volume; CTV: Clinical target volume; PTV: Planning target volume; sup: Bilateral supraclavicular region; min: Minimum; max: Maximum; SD: Standard deviation.

Table 4 Treatment response and toxicities for the 7 patients with cervical and upper thoracic esophageal cancer

Patient No.	Response (primary and node)	Relapse	Life status	Leucopenia (grade)	Dermatitis (grade)	Esophagitis (grade)	Pneumonitis (grade)	Late esophageal toxicity (grade)
1	CR	LR	DOD	1	1	2	0	0
2	Not evaluable	Not evaluable	DOD	1	3	2	0	not evaluable
3	CR	NED	Alive NED	0	1	2	0	0
4	CR	LR	Alive NED	3	1	1	0	3
5	CR	NED	Alive NED	0	3	1	0	3
6	CR	DM and node	DOD	0	3	2	0	0
7	CR	DM	Alive with disease	4	2	4	0	4

LR: Local recurrence in the esophagus; NED: No evidence of disease; DM: Distant metastasis; DOD: Died of disease

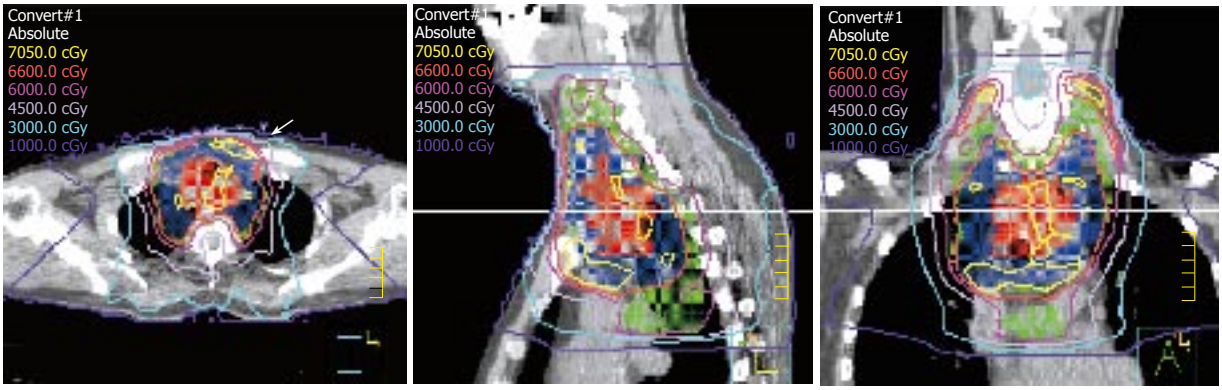


Figure 1 Dose distribution of the IMRT plan for patient number 7. The GTV (red), boost PTV (blue) and PTV (green) are shown on the contours.

treatment are referred as two-phase IMRT strategies. On the other hand, the term "simultaneous integrated boost" (SIB) is used to define treatment plan that delivers differentiated doses to different targets during a single fraction dose delivery as follows: one IMRT plan simultaneously delivers different dose levels to different targets in a single treatment session, which results in the primary target (e.g., palpable or visible disease or GTV) and the secondary target (e.g., regions at risk for microscopic disease or CTV) being treated to different dose levels in each and same treatment fraction. Using this approach, the fractional dose delivered to gross tumor

can be increased, while, at the same time, the radiation doses and dose schedules known to be adequate for tumor control in marginal tissues and clinically uninvolved lymph nodes are preserved. Thus, the SIB-IMRT has been considered a novel method for accelerated fractionation therapy in controlling gross disease through dose-per-fraction escalation. It can shorten overall treatment time, which is preferable for treating tumors with rapid repopulation. It has the ability to deliver large IMRT fields, often required for simultaneous treatments of all target volumes, by splitting them and dynamically feathering their junctions for greater accuracy[23]. Therefore, it is an easy, efficient, and less error-prone method of planning and delivery, because it allows the same plan to be used for the entire course of treatment.

Since the large-field and boost doses are delivered in the same number of fractions, one must consider the radiobiological consequences of different fraction sizes for the gross disease, regions of microscopic spread, and electively treated lymph nodes. One can select the conventional 2 Gy per fraction for the gross disease for an SIB strategy, but that might lead to a significantly lower dose per fraction to volumes of microscopic disease and electively treated lymph nodes. On the other hand, one can choose to deliver 2 Gy per fraction to the lower and intermediate dose volumes, but this would require a high dose per fraction, as much as 2.5 Gy or more per fraction, to the gross disease. The latter scheme may have the advantage of shortening the treatment duration and a potential for improvement in local control but at an increased risk of injury to the embedded normal tissues.

In our study, the IMRT plans showed good homogeneity and conformity and the treatment time was acceptable. SIB-IMRT was used in 5 of 7 patients, which made the treatment course simple. Several researchers reported that SIB-IMRT is superior to two-phase IMRT (sequential IMRT) in terms of conformity of dose distribution within the target volume and the sparing normal tissue for head and neck cancer[24, 25], lung[25,26] and prostate cancers[25]. The SIB-IMRT also has some advantage over 3-dimensional conformal radiotherapy regarding homogeneity of tumor dose and reduction of the dose to normal tissue for cervical esophageal cancer[19] and malignant glioma[27,28].

Defining target volume delineation for IMRT plan, especially a consistent CTV delineation, is challenging because current imaging techniques are not capable of directly detecting subclinical tumor involvement and also because the patterns of failure is not clear. It is difficult to differentiate if the local tumor recurrence is due to a geographic miss or persistent disease. The tendency for esophageal cancer to be multicentric or to present with submucosal skip metastasis supports the use of generous proximal and distal margins for treatment. In the RTOG 8501 trial[6], the entire esophagus was included in the radiation portals, but the toxic effects were severe. In the subsequent RTOG 9405 study[29], 5-cm proximal and distal margins and a 2-cm radial margin were added around the GTV which is effective and has been accepted as a standard in most institutions in the United States.

We used slightly smaller proximal than distal margins in our study, because of our concern about increased toxic effects on the pharynx. Prophylactic irradiation of the supraclavicular and superior mediastinal nodes could decrease the risk of nodal relapse without greatly increasing the toxic effects. In the RTOG 94-05 study[29], boost GTV was defined as a 2-cm margin around the initial GTV; we used the same boost GTV expansion in our study (1.5-cm margin from GTV to boost GTV and another 5 mm added for PTV).

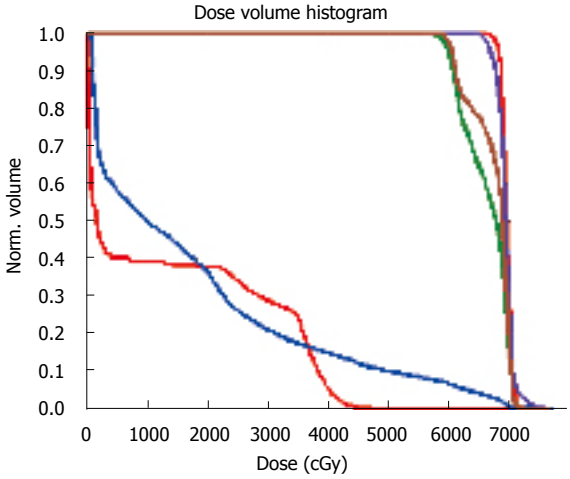


Figure 2 Dose volume histogram (DVH) of the IMRT for patient number 7, showing DVH curves for GTV, boost PTV, CTV, PTV, total lung and spinal cord (from right to left). Norm. Volume = Normalized volume.

This is the first study that we are aware of that reported the clinical result for esophageal cancer patients treated with IMRT. In this study, all 6 evaluable patients achieved complete response both in the primary tumor and lymph node sites. Although the early response result was very encouraging for this small group of locally advanced tumors, 2 patients developed local recurrences and another 2 had distant metastases. Only 3 patients survived with no evidence of disease during a median time of 13 mo follow-up. Although some clinical data on cervical and upper thoracic esophageal cancer patients who were treated with concurrent chemotherapy and radiotherapy with conventional or 3-dimensional conformal techniques have been published, the data are sparse because the disease is rare and the published series have consisted of relatively few patients. For example, Ampil *et al*[7] reported that only 2 of their 6 patients achieved CR when conventional radiation techniques were used to deliver a total dose ranging from 36 to 64 Gy at 2.0 Gy/fraction. Burmeister *et al*[9] reported that 31 of their 34 patients had CR after a total dose delivered by 3-dimensional conformal radiaton that ranged from 50.4 Gy in 20 fractions to 65 Gy in 33 fractions. The 5-year survival rate in this group was 55%. However, only 3 patients in this group had locally advanced disease; most had early disease. Moreover, Bidoli *et al*[8] reported that 37 of their 58 patients (29 of whom had cervical esophageal cancer) experienced a CR after having been given 50 Gy in 25 fractions with conventional radiation techniques. The 10-year survival rate was 27% for the 29 patients with cervical esophageal cancer. We await the maturity of our study to report the long-term outcome.

Three patients experienced severe skin toxicity, probably caused by the use of the mask and the lack of effort to avoid irradiating the skin during IMRT planning. In a study by Burmeister *et al*[9] with 3-dimensional conformal radiotherapy, a moderate-to-severe skin reaction occurred in most patients on the neck. IMRT technique has the potential to reduce skin toxicity if skin was defined as an avoidance structure. From our experience, to reduce toxic effects to the skin, we need to use a cradle instead of a mask to immobilize patient, also consider skin as an avoidance structure during IMRT planning.

The development of acute esophagitis during and after radiotherapy is usually unavoidable. Fortunately, no patient lost more than 10% of body weight or had to have treatment interrupted because of esophagitis, probably because of the intensive supportive and nutritional therapy received through a gastrostomy or jejunostomy tube. Therefore, the prophylactic use of such tubes is recommended for patients who have severe dysphagia or poor nutritional status prior to the initiation of chemoradiation.

Development of late esophageal toxic effects is a concern for esophageal cancer survivors. An esophageal stricture caused by fibrosis of the esophagus, usually at the site of tumor, was mainly due to the high total radiation dose delivered[30]. In this study, 3 of 4 surviving patients had esophageal stricture or fistula. Burmeister *et al*[9] demonstrated that in 34 patients treated with 3-dimensional conformal radiotherapy, strictures were the most notable late effect of the therapy: 11 patients developed mild strictures that did not require dilation, 4 patients developed strictures requiring repeated dilation, and 1 patient developed a severe stricture that did not respond to dilation. In the patient with a severe stricture, the attempted corrective surgery failed and resulted in death of the patient. In addition, the local tumor recurrence remains to be a concern even though relatively high radiation dose was used for this group of patients. The degree of dose escalation by IMRT was limited by long-term toxicity, such as esophageal strictures and fistula. For patients who had their local diseases well-controlled, development of distant metastases became a major pattern of failure.

Therefore, new approaches besides IMRT are needed. One possible way is to use induction chemotherapy. Theoretically, induction chemotherapy could treat the occult micrometastatic disease upfront and may lead to a decrease in the incidence of distant metastases; in addition, induction chemotherapy could be effective in shrinking the primary tumor so that only moderate radiation dose is required to control the local disease, while reducing the late esophageal toxicity. Stuschke *et al*[10] reported their results of induction chemotherapy plus concurrent chemoradiotherapy (a total dose of 60 to 65 Gy in 6 wk delivered by 3D-CRT) for 22 patients with upper and midthoracic locally advanced squamous cell carcinomas. Locoregional recurrences as one site of first relapse were observed in 12 patients and distant metastases as one site of first relapse occurred in 4 patients. Seven long-term survivors had a good swallowing function. Induction chemotherapy results in a major response rate of 45% and response to induction chemotherapy is strongly associated with long-term survival and locoregional tumor control. No long-term survivors are found among non-responders to induction chemotherapy. The criterion good response to induction chemotherapy can be used to select patients for high-dose radiotherapy. Another way is molecular targeting agents. Epidermal growth factor inhibitor (EGFR), such as cetuximab, in combination with radiotherapy yielded positive results in treating head and neck squamous cell carcinoma[31]. It is perhaps worthwhile to test EGFR in treatment of cervical and upper thoracic esophageal cancer, because squamous cell carcinoma is the major histology.

In conclusion, concurrent IMRT and chemotherapy resulted in an encouraging tumor response in patients with locally advanced cervical and upper thoracic esophageal cancer. However, local and distant recurrence, and late toxicity remain to be a challenge in the management of this disease. Additional innovative treatment modalities are needed to improve the outcome. All patients with cervical and upper esophageal tumor should be registered and treated in a prospective study with consistent chemotherapy and IMRT dose schedule, and possibly target therapy.

REFERENCES

1 **Lee DJ**, Harris A, Gillette A, Munoz L, Kashima H. Carcinoma of the cervical esophagus: diagnosis, management, and results. *South Med J* 1984; **77**: 1365-1367

2 **Laterza E**, Mosciaro O, Urso US, Inaspettato G, Cordiano C. Primary carcinoma of the hypopharynx and cervical esophagus: evolution of surgical therapy. *Hepatogastroenterology* 1994; **41**: 278-282

3 **Kelley DJ**, Wolf R, Shaha AR, Spiro RH, Bains MS, Kraus DH, Shah JP. Impact of clinicopathologic parameters on patient survival in carcinoma of the cervical esophagus. *Am J Surg* 1995; **170**: 427-431

4 **Mendenhall WM**, Parsons JT, Vogel SB, Cassisi NJ, Million RR. Carcinoma of the cervical esophagus treated with radiation therapy. *Laryngoscope* 1988; **98**: 769-771

5 **Pearson JG**. The present status and future potential of radiotherapy in the management of esophageal cancer. *Cancer* 1977; **39**: 882-890

6 **Cooper JS**, Guo MD, Herskovic A, Macdonald JS, Martenson JA Jr, Al-Sarraf M, Byhardt R, Russell AH, Beitler JJ, Spencer S, Asbell SO, Graham MV, Leichman LL. Chemoradiotherapy of locally advanced esophageal cancer: long-term follow-up of a prospective randomized trial (RTOG 85-01). Radiation Therapy Oncology Group. *JAMA* 1999; **281**: 1623-1627

7 **Ampil FL**, Mills GM, Burton GV. Induction chemotherapy followed by concomitant chemoradiation for cervical esophageal cancer. *Am J Gastroenterol* 1999; **94**: 2325-2326

8 **Bidoli P**, Bajetta E, Stani SC, De CD, Santoro A, Valente M, Zucali R, Valagussa P, Ravasi G, Bonadonna G. Ten-year survival with chemotherapy and radiotherapy in patients with squamous cell carcinoma of the esophagus. *Cancer* 2002; **94**: 352-361

9 **Burmeister BH**, Dickie G, Smithers BM, Hodge R, Morton K. Thirty-four patients with carcinoma of the cervical esophagus treated with chemoradiation therapy. *Arch Otolaryngol Head Neck Surg* 2000; **126**: 205-208

10 **Stuschke M**, Stahl M, Wilke H, Walz MK, Oldenburg AR, Stüben G, Jahnke K, Seeber S, Sack H. Induction chemotherapy followed by concurrent chemotherapy and high-dose radiotherapy for locally advanced squamous cell carcinoma of the cervical oesophagus. *Oncology* 1999; **57**: 99-105

11 **Mendenhall WM**, Million RR, Bova FJ. Carcinoma of the

cervical esophagus treated with radiation therapy using a four-field box technique. *Int J Radiat Oncol Biol Phys* 1982; **8**: 1435-1439

12 **Mundt AJ**, Mell LK, Roeske JC. Preliminary analysis of chronic gastrointestinal toxicity in gynecology patients treated with intensity-modulated whole pelvic radiation therapy. *Int J Radiat Oncol Biol Phys* 2003; **56**: 1354-1360

13 **Uy NW**, Woo SY, Teh BS, Mai WY, Carpenter LS, Chiu JK, Lu HH, Gildenberg P, Trask T, Grant WH, Butler EB. Intensity-modulated radiation therapy (IMRT) for meningioma. *Int J Radiat Oncol Biol Phys* 2002; **53**: 1265-1270

14 **Vicini FA**, Sharpe M, Kestin L, Martinez A, Mitchell CK, Wallace MF, Matter R, Wong J. Optimizing breast cancer treatment efficacy with intensity-modulated radiotherapy. *Int J Radiat Oncol Biol Phys* 2002; **54**: 1336-1344

15 **Zelefsky MJ**, Fuks Z, Hunt M, Yamada Y, Marion C, Ling CC, Amols H, Venkatraman ES, Leibel SA. High-dose intensity modulated radiation therapy for prostate cancer: early toxicity and biochemical outcome in 772 patients. *Int J Radiat Oncol Biol Phys* 2002; **53**: 1111-1116

16 **Kupelian PA**, Reddy CA, Carlson TP, Altsman KA, Willoughby TR. Preliminary observations on biochemical relapse-free survival rates after short-course intensity-modulated radiotherapy (70 Gy at 2.5 Gy/fraction) for localized prostate cancer. *Int J Radiat Oncol Biol Phys* 2002; **53**: 904-912

17 **Wu VW**, Sham JS, Kwong DL. Inverse planning in three-dimensional conformal and intensity-modulated radiotherapy of mid-thoracic oesophageal cancer. *Br J Radiol* 2004; **77**: 568-572

18 **Nutting CM**, Bedford JL, Cosgrove VP, Tait DM, Dearnaley DP, Webb S. A comparison of conformal and intensity-modulated techniques for oesophageal radiotherapy. *Radiother Oncol* 2001; **61**: 157-163

19 **Fu WH**, Wang LH, Zhou ZM, Dai JR, Hu YM, Zhao LJ. Comparison of conformal and intensity-modulated techniques for simultaneous integrated boost radiotherapy of upper esophageal carcinoma. *World J Gastroenterol* 2004; **10**: 1098-1102

20 **Cox JD**, Stetz J, Pajak TF. Toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC) *Int J Radiat Oncol Biol Phys* 1995; **31**: 1341-1346

21 **Tai P**, Van Dyk J, Yu E, Battista J, Schmid M, Stitt L, Tonita J, Coad T. Radiation treatment for cervical esophagus: patterns of practice study in Canada, 1996. *Int J Radiat Oncol Biol Phys* 2000; **47**: 703-712

22 **Tai P**, Van Dyk J, Yu E, Battista J, Stitt L, Coad T. Variability of target volume delineation in cervical esophageal cancer. *Int J Radiat Oncol Biol Phys* 1998; **42**: 277-288

23 **Wu Q**, Arnfield M, Tong S, Wu Y, Mohan R. Dynamic splitting of large intensity-modulated fields. *Phys Med Biol* 2000; **45**: 1731-1740

24 **Mohan R**, Wu Q, Manning M, Schmidt-Ullrich R. Radiobiological considerations in the design of fractionation strategies for intensity-modulated radiation therapy of head and neck cancers. *Int J Radiat Oncol Biol Phys* 2000; **46**: 619-630

25 **Dogan N**, King S, Emami B, Mohideen N, Mirkovic N, Leybovich LB, Sethi A. Assessment of different IMRT boost delivery methods on target coverage and normal-tissue sparing. *Int J Radiat Oncol Biol Phys* 2003; **57**: 1480-1491

26 **Dirkx ML**, van Sörnsen De Koste JR, Senan S. A treatment planning study evaluating a 'simultaneous integrated boost' technique for accelerated radiotherapy of stage III non-small cell lung cancer. *Lung Cancer* 2004; **45**: 57-65

27 **Chan MF**, Schupak K, Burman C, Chui CS, Ling CC. Comparison of intensity-modulated radiotherapy with three-dimensional conformal radiation therapy planning for glioblastoma multiforme. *Med Dosim* 2003; **28**: 261-265

28 **Thilmann C,** Zabel A, Grosser KH, Hoess A, Wannenmacher M, Debus J. Intensity-modulated radiotherapy with an integrated boost to the macroscopic tumor volume in the treatment of high-grade gliomas. *Int J Cancer* 2001; **96**: 341-349

29 **Minsky BD,** Pajak TF, Ginsberg RJ, Pisansky TM, Martenson J, Komaki R, Okawara G, Rosenthal SA, Kelsen DP. INT 0123 (Radiation Therapy Oncology Group 94-05) phase III trial of combined-modality therapy for esophageal cancer: high-dose versus standard-dose radiation therapy. *J Clin Oncol* 2002; **20**: 1167-1174

30 **Sykes AJ,** Burt PA, Slevin NJ, Stout R, Marrs JE. Radical radiotherapy for carcinoma of the oesophagus: an effective alternative to surgery. *Radiother Oncol* 1998; **48**: 15-21

31 **Bonner JA**, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, Jones CU, Sur R, Raben D, Jassem J, Ove R, Kies MS, Baselga J, Youssoufian H, Amellal N, Rowinsky EK, Ang KK. Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2006; **354**: 567-578

S- Editor Pan BR **L- Editor** Kumar M **E- Editor** Bai SH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5509-5512
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Prevalence of *H pylori* associated 'high risk gastritis' for development of gastric cancer in patients with normal endoscopic findings

Andreas Leodolter, Matthias P Ebert, Ulrich Peitz, Kathlen Wolle, Stefan Kahl, Michael Vieth, Peter Malfertheiner

Andreas Leodolter, Matthias P Ebert, Ulrich Peitz, Kathlen Wolle, Stefan Kahl, Peter Malfertheiner, Department of Gastroenterology and Hepatology, Otto-von-Guericke University Magdeburg, Leipziger Street 44, Magdeburg 39120, Germany
Michael Vieth, Institute of Pathology, Otto-von-Guericke University Magdeburg, Leipziger Street 44, Magdeburg 39120, Germany
Correspondence to: Andreas Leodolter, MD, Department of Gastroenterology and Hepatology, Otto-von-Guericke University, Leipziger Street 44, Magdeburg D-39120, Germany. andreas@Leodolter.de
Telephone: +49-391-6713100 Fax: +49-391-6713105
Received: 2005-09-14 Accepted: 2005-10-26

Abstract

AIM: To investigate the prevalence of *H pylori* associated corpus-predominant gastritis (CPG) or pangastritis, severe atrophy, and intestinal metaplasia (IM) in patients without any significant abnormal findings during upper-GI endoscopy.

METHODS: Gastric biopsies from 3548 patients were obtained during upper GI-endoscopy in a 4-year period. Two biopsies from antrum and corpus were histologically assessed according to the updated Sydney-System. Eight hundred and forty-five patients (mean age 54.8 ± 2.8 years) with *H pylori* infection and no peptic ulcer or abnormal gross findings in the stomach were identified and analyzed according to gastritis phenotypes using different scoring systems.

RESULTS: The prevalence of severe *H pylori* associated changes like pangastritis, CPG, IM, and severe atrophy increased with age, reaching a level of 20% in patients of the age group over 45 years. No differences in frequencies between genders were observed. The prevalence of IM had the highest increase, being 4-fold higher at the age of 65 years versus in individuals less than 45 years.

CONCLUSION: The prevalence of gastritis featuring at risk for cancer development increases with age. These findings reinforce the necessity for the histological assessment, even in subjects with normal endoscopic appearance. The age-dependent increase in prevalence of severe histopathological changes in gastric mucosa, however, does not allow estimating the individual risk for gastric cancer development-only a proper follow-up can provide this information.

Key words: *H pylori*; Gastric cancer; Gastritis

Leodolter A, Ebert MP, Peitz U, Wolle K, Kahl S, Vieth M, Malfertheiner P. Prevalence of *H pylori* associated 'high risk gastritis' for development of gastric cancer in patients with normal endoscopic findings. *World J Gastroenterol* 2006; 12(34): 5509-5512

http://www.wjgnet.com/1007-9327/12/5509.asp

INTRODUCTION

H pylori infection is an established risk factor for development of gastric cancer[1,2]. According to the model of carcinogenesis of the intestinal type adenocarcinoma proposed by Correa, the multi-step development starts from the condition of a chronic active gastritis, followed by glandular atrophy, intestinal metaplasia, dysplasia, and finally gastric adenocarcinoma[3]. The risk for gastric cancer also increases in the presence of corpus-predominant gastritis as well as a pangastritis[4]. Since only a small subset of *H pylori* infected subjects will develop gastric cancer, from a clinical point of view it is a major difficulty to identify those patients with *H pylori* infection who are at a higher risk to develop gastric cancer. Therefore there is a need to identify patients, who have these advanced changes in gastric mucosa. The combination of these risk factors (gastric cancer risk index) has been proven to be a simple way to better estimate the risk for gastric cancer development[5,6]. The gastric cancer risk index has been proven to be independently increased in both histological types of gastric cancer: the intestinal as well as the diffuse type.

The aim of the present study was to estimate the prevalence of advanced histopathological changes in gastric mucosa in patients with normal appearance of gastric mucosa during upper GI-endoscopy.

MATERIALS AND METHODS

A total of 3548 first time upper GI-endoscopies were

Table 1 Age and sex distribution of *H pylori* positive patients without gross pathology of gastric mucosa during upper GI-endoscopy

	Endoscopic normal gastric mucosa with *H pylori* infection
Number (*n*)	845
Mean age (yr)	54.8 (18-87)
Sex	
Male	450/845 (53.2%)
Female	395/845 (46.8%)
Age (yr)	
< 35	114/845 (13.5%)
35-44	120/845 (14.2%)
45-54	151/845 (17.9%)
55-64	199/845 (23.6%)
65-74	166/845 (19.6%)
> 75	95/845 (11.2%)

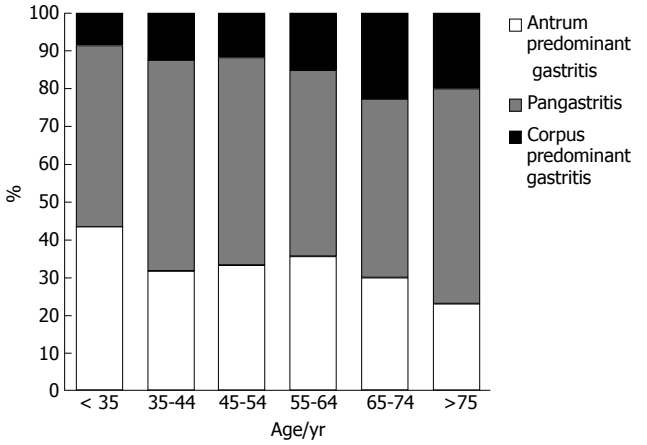


Figure 1 Prevalence of different phenotypes of gastritis in patients with *H pylori* infection in different age groups. With increasing age the number of patients with corpus-predominant gastritis increased significantly, whereas the antrum predominant gastritis decreased (Kruskal Wallis test; $P < 0.01$).

performed in the Endoscopic Unit of the University of Magdeburg over a 4-year period. During this period, it was the policy in our department to obtain gastric specimens from all patients regardless of the appearance of the mucosa to establish the pattern of gastritis in those patients. Patients with contraindication to obtain gastric specimens or previous endoscopy with histological investigation were not included.

Around one half of the investigated patients were outpatients, and the other half hospitalized patients. After the investigation, an endoscopic report was entered in a computer-based database. Upper GI-endoscopy was performed by several experienced gastroenterologists. Two gastric specimens from the corpus and antrum, respectively, were taken and analysed histologically according to the Sydney-System in all patients. Histological results were documented in the same computer-based database. Histological examination was performed by different pathologists from the Institute of Pathology at the University of Magdeburg. All slides have been re-evaluated for the needs of this study by a single experienced gastrointestinal pathologist in a blinded way without being aware of clinical or endoscopic diagnoses and prior histological reports. The study was performed according to the guidelines of the Ethics Committee of the Medical Faculty of the University of Magdeburg and all patient data were made anonymous to protect patients' identity.

The database was retrospectively analyzed for those patients who had a normal endoscopical appearance of gastric mucosa or simply signs of gastritis or duodenitis. Thus all patients with gastric or duodenal ulcers, GI-bleeding, and upper GI-malignancy were excluded. The presence of reflux oesophagitis or hiatal hernia was not an exclusion criterion. Furthermore, patients known to be treated with proton-pump inhibitors or with indirect evidence for this treatment by histological observation of parietal cell hypertrophy were excluded. *H pylori* infection was determined by positive culture, rapid urease test and/or histology.

We identified 845 *H pylori* positive patients who fulfilled the predefined criteria. Age distribution and gender are given in Table 1. In these patients the following parameters were calculated according to the histological examination: (1) Corpus-predominant gastritis[4]: Higher degree of neutrophilic infiltration in the corpus compared to the antrum; (2) Pangastritis[4]: Equal degree of neutrophilic infiltration in the corpus and in the antrum; (3) Antrum-predominant gastritis[4]: Higher degree of neutrophilic infiltration in the antrum compared to the corpus; (4) Intestinal metaplasia[4,7]: Absence or presence in any investigated specimen from antrum or corpus; (5) Severe atrophy[4,8]: Severe loss of glands; not diagnosed in the antrum when only a few intestinal crypts were observed in the whole specimen; (6) Antrum and corpus gastritis score: Score by total sum of grade of gastritis (mild = 1, moderate = 2, marked = 3 infiltration with lymphocytes and plasma cells) and activity of gastritis (mild = 1, moderate = 2, marked = 3 infiltration with neutrophilic granulocytes) either in the antrum or in the corpus, a maximum of a sum of 6 points in the antrum and in the corpus for each individual person; (7) Gastric cancer risk index[5,6]: 1 point scored for at least moderate infiltration with lymphocytes and plasma cells in the corpus and less or equal infiltration in the antrum, 1 point scored for at least moderate infiltration with neutrophilic granulocytes in the corpus and less or equal infiltration in the antrum, 1 point scored for the detection of intestinal metaplasia in the antrum or in the corpus, a maximum of 3 points for each individual person.

RESULTS

The frequency of corpus-predominant gastritis was significantly increased with age (Kruskal-Wallis test, $P < 0.01$). While in the group of patients of less than 35 years old only 8.8% had a corpus-predominant gastritis, and the number increased to 22.9% between the ages of 65-75 years (Figure 1). The highest increase was observed between the age groups of 45-54 years (13.8%) and 55-64 years (23.1%). The number of patients with pangastritis remained constant, whereas the frequency of antrum-predominant gastritis declined over time.

Table 2 Frequency of intestinal metaplasia (IM) in the gastric antrum and the corpus in patients without macroscopic pathology during upper GI-endoscopy

Age (yr)	Overall IM	IM only in the antrum	IM only in the corpus	IM in antrum and corpus
< 35	9/114 (7.9%)	1/114 (0.9%)	7/114 (6.1%)	1/114 (0.9%)
35-44	14/120 (11.7%)	2/120 (1.7%)	12/120 (10.0%)	0/120 (0.0%)
45-54	28/151 (18.5%)	7/151 (4.6%)	18/151 (11.9%)	3/151 (2.0%)
55-64	50/199 (25.1%)	7/199 (3.5%)	35/199 (17.6%)	8/199 (4.0%)
64-75	65/166 (39.2%)	6/166 (3.6%)	51/166 (30.7%)	8/166 (4.8%)
> 75	38/95 (40.0%)	6/95 (6.3%)	24/95 (25.2%)	8/95 (8.4%)
Differences between age groups	$P < 0.001$ (χ^2-test)	$P < 0.001$ (χ^2-test)	$P < 0.001$ (χ^2-test)	$P < 0.001$ (χ^2-test)

Table 3 Frequency of severe atrophy, antrum- and corpus-gastritis score in *H pylori* positive patients in different age-groups in patients without macroscopic pathology during upper GI-endoscopy

Age (yr)	Severe atrophy	Antrum gastritis score	Corpus gastritis score
< 35	0/114 (0.0%)	3.36 ± 0.90	2.49 ± 1.17
35-44	2/120 (1.7%)	3.17 ± 0.86	2.73 ± 1.02
45-54	3/151 (2.0%)	3.32 ± 1.04	2.85 ± 1.04
55-64	4/199 (2.0%)	3.38 ± 0.94	2.93 ± 1.17
64-75	9/166 (5.4%)	3.20 ± 0.92	2.98 ± 1.08
> 75	9/95 (11.2%)	3.08 ± 0.96	3.06 ± 1.15
Differences between age groups	$P < 0.001$ (χ^2-test)	NS (ANOVA)	$P < 0.001$ (ANOVA)

Table 4 Comparison of gastric cancer risk index and phenotype of gastritis in 845 patients with *H pylori* positive gastritis

Phenotype of gastritis	Gastric cancer risk index			
	0 point	1 point	2 points	3 points
Antrum-predominant gastritis	156	104	21	0
Pangastritis	107	178	113	36
Corpus-predominant gastritis	2	22	62	44

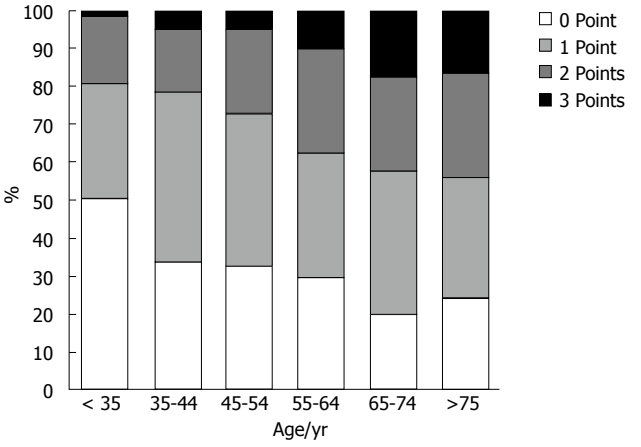


Figure 2 Gastric cancer risk index in patients with *H pylori* infection in different age groups. With increasing age the number of patients with 2 or 3 points increased significantly (Kruskal Wallis test; $P < 0.0001$).

The frequencies of intestinal metaplasia in different age groups increased significantly in an almost linear pattern ($r^2 = 0.9585$, $P < 0.001$). The increase rate was 0.71% per year (95% CI 0.51-0.92). Nearly 40% of patients over 65 years had intestinal metaplasia (Table 2). For example, the relative risk for a patient at the age of 64-75 years was 5.0 (95% CI 2.6-9.6) times higher compared to a patient younger than 35 years. Intestinal metaplasia was more frequent in the corpus compared to the antrum (20.7% *vs* 6.7%), and those located only in the antrum were a rare event (3.4%).

The frequencies of severe atrophy and the antrum/corpus gastritis score in the different age groups are provided in Table 3. Severe atrophy was close to 2% in the group younger than 65 years and increased up to 11.2% at the age over 75 years. The antrum gastritis score was almost equal in all age groups (mean 3.27 ± 0.95). The corpus gastritis score increased significantly with age [increase of 0.01 per year (95% CI 0.006-0.016)].

The gastric cancer risk index showed a significant shift to a higher index with age (Kruskal-Wallis test; $P < 0.0001$; Figure 2). At least 10% of the individuals older than 55 years had a score of 3 points, and more than 25% of them had a score of 2 points.

Comparison of the different scoring systems like gastric cancer index and phenotype of gastritis showed a significant correlation ($P < 0.001$, χ^2-test; Table 4). This was expected because the phenotype of gastritis was included in the gastric cancer index. Nevertheless, the definitions for predominant gastritis were very different (see METHODS). The group with the highest risk for gastric cancer according to Uemura *et al*[4] might be these with the corpus-predominant gastritis ($n = 130$), whereas the risk might be highest for those with a gastric cancer index of 3 ($n = 80$), and the overlapping group contained only 44 patients (26.5%).

DISCUSSION

Histological alterations of the gastric mucosa such as corpus-predominant gastritis, pangastritis, intestinal metaplasia or severe atrophy are proposed to carry an increased risk for gastric cancer in *H pylori* infected individuals. In our study we observed a strong association of intestinal metaplasia, atrophy as well as corpus-predominant gastritis with increasing age. Our results represent the frequency of histopathological changes in a country with a lower gastric cancer prevalence compared to other parts of the world (i.e. China or Japan). This might be of importance for future investigational trials on this topic.

We investigated only patients with normal appearing gastric mucosa or no grossly visible changes at endoscopy. In such a setting, often a rapid urease test would be performed by most investigators to test for *H pylori* only; therefore histopathological changes will not be diagnosed. However, our data clearly indicate that there is a need to obtain gastric specimens in routine endoscopy, even if the macroscopic appearance is to a greater or lesser extent normal. There is much evidence that several phenotypic changes in the gastric mucosa are associated with an

increased risk of gastric cancer. In a large prospective study from Japan[4] only patients with *H pylori* infection developed gastric cancer. A closer look at the data revealed that certain histopathological findings increased the risk dramatically. In fact, the presence of intestinal metaplasia (RR 6.4), severe atrophy (RR 4.9), pangastritis (RR 15.6), and, most strikingly, corpus-predominant gastritis (RR 34.5) was associated with an increased risk for gastric cancer. An additional aspect in this study was the fact that about 60% of the patients who developed gastric cancer, had no initial pathological findings during upper GI-endoscopy and were classified as non-ulcer dyspepsia. It demonstrates the importance to obtain gastric specimen during upper GI-endoscopy even though the macroscopic findings are normal.

The optimal strategy for patients with risk lesions for gastric cancer has not been established. One option might be to eradicate *H pylori* infection. It is well known that corpus-predominant or pangastritis will be healed after successful eradication therapy; on the other hand, atrophy and intestinal metaplasia will persist constantly according to systematic review of the published literature[9]. In addition, a large randomized trial from China indicates that the point of no return might be already achieved when atrophy or intestinal metaplasia are observable[10]. In that trial, performed in more than 1600 healthy volunteers from Fujian Province, a significant reduction of gastric cancer incidence in an 8-year follow-up period by *H pylori* eradication was only observed in those patients without intestinal metaplasia or atrophy in the gastric mucosa in the beginning of the study. One possible conclusion from this study is that patients with those histopathological changes have to be included in an endoscopic surveillance program, but it is unknown which intervals are necessary and how cost-effective such a strategy might be. This will have to be the topic of future investigational trials.

The combination of histopathological features is a promising tool, since there is a clear need to identify those patients at risk for the development of gastric cancer. While the scoring system used in the trial by Ley *et al*[11] is probably too complex to use in general, the gastric cancer index might be more promising to use in clinical practice. This score includes the type of gastritis as well as the presence of intestinal metaplasia and has been proven to be highly predictive for the presence of gastric carcinoma[5]. We identified 80 (9.5%) out of 845 patients with normal endoscopic appearance and the highest possible gastric cancer risk score of 3. Especially in those patients an endoscopic surveillance might be necessary; however, the final proof of any improvement for the individual patient is yet missing.

In the trial of Uemura *et al*[4] the highest risk was found in those patients with a corpus-predominant gastritis; nevertheless, a marked risk was observed in those with pangastritis, also. Pangastritis was very frequently diagnosed in our population (51.3%). The significance of this finding therefore will have to be verified.

Our results indicate that there is a need to obtain gastric biopsies during upper GI-endoscopy, even if the macroscopic appearance is normal or without any gross pathology. The prevalence of gastritis associated with an increased risk for cancer development increases with age. The individual risks for gastric cancer development remain to be established by the follow-up. *H pylori* eradication therapy alone might not be sufficient enough to prevent gastric cancer in patients with advanced changes in the gastric mucosa. From the actual perspective, and with regard of the literature, interventions have to be implemented earlier, before advanced changes in gastric mucosa caused by *H pylori* occur or, more worthwhile, preventing being infected with *H pylori*.

REFERENCES

1 **Parsonnet J**, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131

2 **Forman D**, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sitas F. Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. *BMJ* 1991; **302**: 1302-1305

3 **Correa P**. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992; **52**: 6735-6740

4 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789

5 **Meining A**, Kompisch A, Stolte M. Comparative classification and grading of Helicobacter pylori gastritis in patients with gastric cancer and patients with functional dyspepsia. *Scand J Gastroenterol* 2003; **38**: 707-711

6 **Meining A**, Bayerdörffer E, Müller P, Miehlke S, Lehn N, Hölzel D, Hatz R, Stolte M. Gastric carcinoma risk index in patients infected with Helicobacter pylori. *Virchows Arch* 1998; **432**: 311-314

7 **Meining AG**, Bayerdörffer E, Stolte M. Helicobacter pylori gastritis of the gastric cancer phenotype in relatives of gastric carcinoma patients. *Eur J Gastroenterol Hepatol* 1999; **11**: 717-720

8 **Heikkinen M**, Vornanen M, Hollmén S, Färkkilä M. Prognostic significance of antrum-predominant gastritis in functional dyspepsia. *Scand J Gastroenterol* 2004; **39**: 227-231

9 **Hojo M**, Miwa H, Ohkusa T, Ohkura R, Kurosawa A, Sato N. Alteration of histological gastritis after cure of Helicobacter pylori infection. *Aliment Pharmacol Ther* 2002; **16**: 1923-1932

10 **Wong BC**, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, Ching CK, Chen JS. Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 2004; **291**: 187-194

11 **Ley C**, Mohar A, Guarner J, Herrera-Goepfert R, Figueroa LS, Halperin D, Johnstone I, Parsonnet J. Helicobacter pylori eradication and gastric preneoplastic conditions: a randomized, double-blind, placebo-controlled trial. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 4-10

S- Editor Wang GP **L- Editor** Zhu LH **E- Editor** Bi L

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5513-5516
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Genetic association of autoimmune hepatitis and human leucocyte antigen in German patients

Andreas Teufel, Markus Wörns, Arndt Weinmann, Catherine Centner, Anja Piendl, Ansgar W Lohse, Peter R Galle, Stephan Kanzler

Andreas Teufel, Markus Wörns, Arndt Weinmann, Catherine Centner, Anja Piendl, Peter R Galle, Stephan Kanzler, Department of Medicine I, Johannes Gutenberg University, Mainz, Germany
Ansgar W Lohse, Department of Medicine I, University of Hamburg, Germany
Correspondence to: Stephan Kanzler, MD, Department of Internal Medicine I, Johannes Gutenberg University, Building 301, Langenbeckstr. 1, Mainz 55101, Germany. kanzler@mail.uni-mainz.de
Telephone: +49-61-31173764 Fax: +49-61-31175222
Received: 2005-12-10 Accepted:2006-01-24

Abstract

AIM: To report on our large German collective and updated data of 142 patients with autoimmune hepatitis (AIH) type 1.

METHODS: Key investigations performed were liver biopsy, serum autoantibodies as well as serum markers such as IgG and elevated transaminases. Antinuclear antigen (ANA) and smooth muscle antigen (SMA) autoantibodies characterized type 1 AIH. Type 3 (AIH) was solely characterized by the occurrence of soluble liver antigen/liver-pancreas antigen (SLA/LP) autoantibodies either with or without ANA or SMA autoantibodies.

RESULTS: Most prevalent HLAs were A2 (68 patients, 48%), B8 (63 patients, 44%), C7 (90 patients, 63%), DR3 (49 patients, 38%), DR4 (49 patients, 38%) and DQ2 (42 patients, 30%). Compared to the Italian and North American patients, we found fewer patients with a DQ2 subtype. Furthermore, the B8-DR3-DQ2 human leucocyte antigen (HLA) was also less prominent compared to the North American patients. However, prevalences of B8, DR3, DR4, DR7, DR11 and DR13 were comparable to the Italian and North American patients. Furthermore, we report on an additional subgroup of patients with SLA/LP positive AIH. Generally, in this subgroup of patients the same HLA subtypes were favoured as the AIH type 1.

CONCLUSION: Although HLA subtypes were comparable between these three collectives, the German patients were distinct from the Italian and North American patients with respect to DQ2 and from the North American patients with respect to B8-DR3-DQ2 HLA. A clinical correlation, e.g. difference in severity or treatability of AIH type 1, has yet to be determined.

Key words: Autoimmune hepatitis; Human leucocyte antigen; Immunogenetics

Teufel A, Wörns M, Weinmann A, Centner C, Piendl A, Lohse AW, Galle PR, Kanzler S. Genetic association of autoimmune hepatitis and human leucocyte antigen in German patients. *World J Gastroenterol* 2006; 12(34): 5513-5516

http://www.wjgnet.com/1007-9327/12/5513.asp

INTRODUCTION

Autoimmune hepatitis (AIH) is characterized by portal lymphatic infiltrates on liver histology and in most patients with the occurrence of autoantibodies such as antinuclear, smooth muscle antibody-positive (ANA/SMA, type 1), liver-kidney microsomal antibody-positive (LKM-1), and soluble liver antigen/liver-pancreas antigen (SLA/LP) antibodies. Untreated, the disease usually runs an unfavorable course with 5 year survival rates of 50% and 10 year survival rates between 10% and 27%[1,2]. However, in most patients the disease can be controlled with immunosuppressants such as prednisolone and azathioprine, resulting in an almost normal average life expectancy[3].

Although the pathomechanism of the disease is still unknown, an underlying genetic predisposition has been suggested due to the fact that patients are predominantly of female gender (women to men ratio equals to approximately 6:1) and the association of the disease with certain human leucocyte antigens (HLAs). Muratori *et al*[4] recently published an extensive study on two large populations, Italian and North American, and demonstrated a distinct genetic association of HLA with the disease. B8-DR3-DQ2 was reported to be the most frequent genotype in Italian patients with AIH type 1 but significantly less frequent in North American patients. In addition, a clear difference in occurrence of the DR4 genotype was demonstrated with fewer patients in Italy presenting with such a genotype. Furthermore, the C7, DR3, DR11, DR13, and DQ2 loci were investigated but no significant differences between

Table 1 Most prevalent HLA subtypes in German patients with autoimmune hepatitis type 1

HLA	AIH type I German patients
C7	90 (63%)
A2	68 (48%)
B8	63 (44%)
DR3	49 (38%)
DR4	49 (38%)
DQ2	42 (41%)

these two populations could be found.

In order to further elucidate these genetic associations and differences, we now report on the HLA antigens in our large German collective of 142 patients with AIH type 1 and compared it to published data, especially in the Italian and North American populations. In addition, we report on our small subgroup of 29 patients with SLA/LP positive AIH.

MATERIALS AND METHODS

Subjects

One hundred and forty-two consecutive patients with definite, autoantibody positive AIH type 1 and 29 consecutive patients with SLA/LP positive AIH, who had been referred to the Department of Internal Medicine I, Mainz University Hospital, were investigated. All patients lacked serological evidence of chronic viral hepatitis B and C by third-generation enzyme-linked immunoassay. There was no evidence for illicit drug abuse, excessive alcohol intake (> 4 oz/wk) or exposure to hepatotoxic drugs. Diagnosis of AIH was established according to the revised Scoring system described previously[5].

Methods

Key investigations performed were liver biopsy, serum autoantibodies as well as serum markers such as IgG and elevated transaminases. Similar to Muratori's study[4] and according to international standards, ANA and SMA autoantibodies characterized type 1 AIH. Type 3 (AIH) was solely characterized by the occurrence of SLA/LP autoantibodies either with or without ANA or SMA autoantibodies.

Of our 142 patients with AIH type 1, 119 were women (84%) and 23 were men (16%). Of these patients, 108 (76%) were positive for ANA, 101 (71%) for SMA autoantibodies, and 67 (47%) patients had both ANA and SMA autoantibodies. DR and DQ alleles were not examined in all patients. DR alleles were investigated in 129 patients and DQ in 103 patients. Of the 29 patients with AIH type 3, 22 were women (76%) and 7 were men (24%). Since HLA loci are thought to be genetically fixed and to be independent of age, serum markers or immune globulins, patients were not further characterized in that respect.

RESULTS

The most commonly found HLA subtypes in our patients with AIH type 1 were C7 (90 patients, 63%), A2 (68 patients, 48%), B8 (63 patients, 44%), DR3 (49 patients, 38%), DR4 (49 patients, 38%) and DQ2 (42 patients, 30%) (Table 1). As significant differences had been demonstrated in the Italian and North American populations regarding the distribution of the B8-DR3-DQ2 and DR4 HLA subtypes, these subtypes were also analysed along with the additionally reported C7, DR7, DR11, DR13, and DQ2 loci. The B8-DR3-DQ2 subtype was identified in 28 (27%) German patients with AIH type 1. And 49 patients (38%) were tested positive for the DR4 locus. In addition, almost half of all patients with AIH type 1 were positive for the HLA subtype B8 (45%) and 38% for DR3 (Table 2). The C7 HLA subtype which was highly prevalent in Italian patients with AIH type 1 was also common in the German population with 90 patients (63%). Further HLA subtypes reported and compared in Italian and North American patients were less prevalent in the German population. DR11 was positive in 17 (13%) of patients. Also DR13 could be identified in 17 (13%) of patients. Compared to the North American and Italian patients the DQ2 allele was also less prominent with 42 (41%) of patients.

For patients with SLA/LP positive AIH, HLA association had previously not been reported. Thus, we assessed the association of HLA of 29 patients with SLA positive, type 3 AIH (Table 3). Given a significantly smaller collective, there seemed to be a trend for SLA/LP positive AIH to be associated with the same HLA as AIH type 1. The most prevalent HLA in our patients with AIH were A2 in 16 patients (55%), B8 in 15 patients (52%), C7 in 22 patients (76%), DR3 in 12 patients (41%), and DQ2 in 13 patients (45%). Interestingly, only 3 patients did not carry either DR3 or DR4.

DISCUSSION

The etiology of AIH is still unknown. However, an underlying genetic predisposition has been suggested due to the fact that patients are predominantly of female gender and the association of the disease with certain HLA. Muratori *et al*[4] recently published an extensive study on two large populations, Italian and North American, and demonstrated a distinct genetic association of HLA antigens with the disease. Since this study not only investigated the commonly studied HLA antigens type Ⅱ but also extensively investigated the HLA antigens type Ⅰ, this study was of significant value. In order to further elucidate HLA association with AIH and to compare our collective of patients with AIH, which is among the largest reported, to published populations in different regions of the world, we here present our data on HLA association in German patients.

Within the Italian population significant association of AIH was demonstrated for HLA antigens B8 (32%), C7 (51%), DQ2 (53%) and for the combined HLA type B8-DR3-DQ2 (30%). The frequencies of B8 and C7 were confirmed by our study (45% and 63%). In addition, an earlier British study had also reported on an association of increased frequency of C7 with AIH type 1[6]. In contrast, DQ2 was found less prominent in German patients compared to Italian (41% *vs* 53%) and North American

Table 2 Prevalence of HLA subtypes in German patients with autoimmune hepatitis type 1 and comparison to worldwide populations

HLA	Germany *n* = 142	Italy[5] *n* = 57	N. America[5] *n* = 149	N. America II[8] *n* = 161	UK[6] *n* = 87	West India[10] *n* = 20	Japan[7] *n* = 77	China[9] *n* = 32	Brazil[8] *n* = 115	Turkey[11] *n* = 17
A2	48%					16%				
B8	45%	32%	49%			3%				
C7	63%	51%			70%	29%				
DR3	38%	30%	52%	47%		11%	31%	16%	32%	17%
DR4	38%	23%	43%	45%		3%	83%	50%	16%	59%
DR7	16%	16%	15%							
DR11	13%	18%	7%							
DR13	13%	26%	16%							
DQ2	41%	53%	57%							
B8-DR3-DQ2	27%	30%	48%							

(41% *vs* 57%) patients, but still at a higher frequency compared to the Italian control population (30%). Finally, the combined HLA subtype B8-DR3-DQ2 was less frequent in German patients, especially compared to North American patients (27% *vs* 48%). This may mostly be due to the significant lower frequency of the DQ2 HLA in German patients.

On the contrary, our patients displayed DR4 HLA frequency that was highly similar to the North American patients and significantly higher than the reported Italian HLA associations. An association of DR4 with AIH type 1 had been reported and in other populations, especially in Japan (83%) and Brazil (50%) it had an even higher frequency[7,8].

Frequency of HLA DR3 was highly similar between German and North American patients (45% and 52%), which were clearly higher than in all other populations reported thus far. Comparable to Italian patients DR3 was found at 31% in Japanese and 32% in Brazilian patients. In smaller populations from Western India, China and Turkey, this HLA antigen was even found at 11% to 17%[9-11]. Moreover, 49 of all patients carried neither DR3 nor DR4. However, a search for highly prevalent HLA in these patients did not reveal any additional, obvious new association, independent of DR3 or DR4.

All other HLA frequencies, specifically reported and compared by Muratori were comparable between our collective and the Italian and the North American study groups. The results of all three study groups are summarized in Table 2.

To date, most studies on HLA association with AIH mainly focused on AIH type 1 patients and reported only a few AIH type 2 patients. Thus far, HLA association of SLA/LP positive AIH has not been reported in a significant number of patients. Therefore, we extended our analysis of HLA antigen association to SLA/LP positive AIH. Given a smaller group of only 29 patients, HLA frequencies seemed to be similar to patients with AIH type 1. Comparable to AIH type 1, a high frequency of C7 (77%) was found. Frequencies for A2, B8, and DR3 were comparable to those of our German patients with AIH type 1 but also to AIH type 1 patients of the Italian and North American groups, who had not reported data on their AIH type 3 patients. Clearly a higher frequency of HLA DQ2 was observed in patients with SLA/LP positive AIH compared to our patients with AIH type 1. However, these higher HLA DQ2 frequencies are comparable to the DQ2 frequencies of the Italian and North American groups. Together, these data suggest a common genetic association and background for AIH types 1 and 3. This is in accordance with the observation that type 1 and SLA/LP positive AIH are also comparable with respect to their clinical course[2].

Table 3 Most prevalent HLA subtypes in German patients with SLA/LP positive autoimmune hepatitis

HLA	AIH SLA/LP positive German patients *n* = 29
A2	16 (55%)
B8	15 (52%)
C7	22 (76%)
DR3	12 (41%)
DQ2	13 (45%)

In conclusion, German AIH type 1 patients were demonstrated to be genetically distinct from Italian or North American patients and other populations, especially with respect to a significantly lower frequency of HLA DQ2 and a lower occurrence of HLA B8-DR3-DQ2. Other HLAs were found at similar frequencies, suggesting an underlying genetic background of AIH type 1. Analysis of a smaller group of 29 patients with SLA/LP positive AIH pointed towards comparable HLA frequencies in patients with AIH type 1 and SLA/LP positive AIH, which is in accordance with a similar clinical course. The challenge is yet to investigate whether these findings may help to better understand the etiology of AIH, to predict prognosis of the disease, or to further improve therapeutic concepts.

REFERENCES

1 **Johnson PJ**, McFarlane IG, Eddleston AL. The natural course and heterogeneity of autoimmune-type chronic active hepatitis. *Semin Liver Dis* 1991; **11**: 187-196

2 **Kanzler S**, Löhr H, Gerken G, Galle PR, Lohse AW. Long-term management and prognosis of autoimmune hepatitis (AIH):

a single center experience. *Z Gastroenterol* 2001; **39**: 339-341, 344-348

3 **Kanzler S**, Gerken G, Löhr H, Galle PR, Meyer zum Büschenfelde KH, Lohse AW. Duration of immunosuppressive therapy in autoimmune hepatitis. *J Hepatol* 2001; **34**: 354-355

4 **Muratori P**, Czaja AJ, Muratori L, Pappas G, Maccariello S, Cassani F, Granito A, Ferrari R, Mantovani V, Lenzi M, Bianchi FB. Genetic distinctions between autoimmune hepatitis in Italy and North America. *World J Gastroenterol* 2005; **11**: 1862-1866

5 **Alvarez F**, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, Chapman RW, Cooksley WG, Czaja AJ, Desmet VJ, Donaldson PT, Eddleston AL, Fainboim L, Heathcote J, Homberg JC, Hoofnagle JH, Kakumu S, Krawitt EL, Mackay IR, MacSween RN, Maddrey WC, Manns MP, McFarlane IG, Meyer zum Büschenfelde KH, Zeniya M. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol* 1999; **31**: 929-938

6 **Strettell MD**, Thomson LJ, Donaldson PT, Bunce M, O'Neill CM, Williams R. HLA-C genes and susceptibility to type 1 autoimmune hepatitis. *Hepatology* 1997; **26**: 1023-1026

7 **Yoshizawa K**, Ota M, Katsuyama Y, Ichijo T, Matsumoto A, Tanaka E, Kiyosawa K. Genetic analysis of the HLA region of Japanese patients with type 1 autoimmune hepatitis. *J Hepatol* 2005; **42**: 578-584

8 **Czaja AJ**, Souto EO, Bittencourt PL, Cancado EL, Porta G, Goldberg AC, Donaldson PT. Clinical distinctions and pathogenic implications of type 1 autoimmune hepatitis in Brazil and the United States. *J Hepatol* 2002; **37**: 302-308

9 **Qiu DK**, Ma X. Relationship between human leukocyte antigen-DRB1 and autoimmune hepatitis type I in Chinese patients. *J Gastroenterol Hepatol* 2003; **18**: 63-67

10 **Shankarkumar U**, Amarapurkar DN, Kankonkar S. Human leukocyte antigen allele associations in type-1 autoimmune hepatitis patients from western India. *J Gastroenterol Hepatol* 2005; **20**: 193-197

11 **Kosar Y**, Kacar S, Sasmaz N, Oguz P, Turhan N, Parlak E, Heneghan MA, McFarlane IG. Type 1 autoimmune hepatitis in Turkish patients: absence of association with HLA B8. *J Clin Gastroenterol* 2002; **35**: 185-190

S- Editor Wang GP **L- Editor** Zhu LH **E- Editor** Bai SH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5517-5522
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Expression pattern of leptin and leptin receptor (OB-R) in human gastric cancer

Makoto Ishikawa, Joji Kitayama, Hirokazu Nagawa

Makoto Ishikawa, Joji Kitayama, Hirokazu Nagawa, Department of Surgery, Division of Surgical Oncology, the University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
Correspondence to: Makoto Ishikawa, Department of Surgical Oncology, Faculty of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. makoto-ishi@umin.ac.jp
Telephone: +81-3-38155411-33246 Fax: +81-3-38116822
Received: 2005-07-29 Accepted: 2005-08-26

Abstract

AIM: To examine the expression of leptin and its receptor, OB-R, in normal gastric mucosa and neoplasia.

METHODS: By immunohistochemical staining using specific antibodies, we evaluated the expression of leptin and OB-R in 207 gastric carcinomas (100 early and 107 advanced carcinomas) and analyzed their relationship with clinicopathological features.

RESULTS: Both normal gastric epithelium and carcinoma cells expressed a significant level of leptin. In cases with OB-R staining, carcinoma cells showed OB-R-positive expression, but the intensity was weaker than that in normal mucosa. The expression of OB-R showed a significant correlation with the level of leptin expression. The expression levels of both leptin and OB-R tended to increase as the depth of tumor invasion or TMN stage increased ($P < 0.01$). Lymph node metastasis was detected in 49.5% (47/95) of leptin-strong cases and in 50.5% (48/95) of OB-R-positive cases, and the rate was 33% (37/112) in leptin-weak cases and 17% (19/112) in OB-R-negative cases. Both venous and lymphatic invasion also tended to be observed frequently in positive tumors as compared with negative tumors. Interestingly, in the 96 leptin- or OB-R-positive tumors, hematogenous metastasis was detected preoperatively in 3 (3.1%) patients. In contrast, none of the carcinomas that lacked expression of leptin and OB-R showed hematogenous metastasis.

CONCLUSION: Overexpression of leptin and expression of OB-R may play a positive role in the process of progression in gastric cancer. Functional upregulation of leptin/OB-R may have a positive role in the development and initial phase of progression in gastric cancer.

Key words: Leptin; OB-R; Gastric cancer

Ishikawa M, Kitayama J, Nagawa H. Expression pattern of leptin and leptin receptor (OB-R) in human gastric cancer. *World J Gastroenterol* 2006; 12(34): 5517-5522

http://www.wjgnet.com/1007-9327/12/5517.asp

INTRODUCTION

Previous studies have shown a positive association between adiposity and increased risk of cancers of the endometrium, kidney, gallbladder (in women), breast (in postmenopausal women), and colon (particularly in men)[1-9]. Consistently, a recent large scale cohort study demonstrated that increased body weight is associated with increased mortality for all cancers combined, as well as for cancers at various specific sites[10].

The adipocyte-derived cytokine, leptin, is thought to play a key role in the control of satiety, energy expenditure, and various reproductive processes[11-13]. Leptin is a peptide hormone composed of 167 amino acids with a molecular mass of 16 kDa[14]. Generally, plasma leptin level is representative of body fat mass[15-18] and increases in a logarithmic fashion with an increase in body mass in mice[19]. Leptin controls body mass and metabolism by affecting the metabolic, neuroendocrine, reproductive and hematopoietic systems[20]. In cancer, there is regulatory dysfunction in metabolic, neuroendocrine and other systems. Although initially thought to be exclusively expressed in and secreted by adipocytes, recent studies have shown that leptin expression can be detected in a number of additional tissues, including the placenta[21], gastric mucosa[22], pituitary cells[23] and hepatic stellate cells[24]. More importantly, leptin has been shown to regulate cell proliferation in diverse normal and malignant tissues and to stimulate the proliferation of certain normal hematopoietic and epithelial cells. Leptin has also been shown to promote the invasiveness of premalignant colon and kidney epithelial cells *in vitro*[25]. These findings suggest the possibility that leptin may be critically involved in the promotion of cancer.

Leptin exerts its action through the leptin receptor (OB-R), a member of the cytokine family of receptors, which was also detected in both rat and human gastric mucosa. Further studies, however, have shown that leptin receptors are expressed in many other tissues, including

the brain, placenta, pancreas, adrenal gland, hematopoietic cells, liver, lung and heart[21,24,26-28]. In addition, OB-R has been identified in malignant cells, including lung and gastric carcinoma and leukemic cells[22,29-32].

In this study, therefore, we used antibodies to leptin and OB-R, and immunohistochemically characterized the expression pattern of these two proteins in gastric carcinoma and evaluated the possible role of leptin in the tumorigenesis and spread of gastric cancer.

MATERIALS AND METHODS

Two hundred and seven carcinomas, which were surgically resected in the Department of Surgery, The University of Tokyo, from 1991 to 2002, were included in this study. In all cases, serial-step sections 3-mm wide were cut, fixed in 10% formalin solution, and then embedded in paraffin. All the resected primary tumors and regional lymph nodes were histologically examined by hematoxylin-eosin staining according to the Japanese Classification of Gastric Carcinoma[33]. Tumors were histologically classified into two types based on the predominant features: differentiated type (well and moderately differentiated adenocarcinoma) and undifferentiated type (poorly differentiated adenocarcinoma and signet ring cell carcinoma). In addition, we examined several discrete histological parameters, including lymphatic invasion, venous invasion and lymph node metastasis.

Immunohistochemical study of leptin and OB-R

We investigated the expression of OB-R and leptin with immunohistochemical staining using affinity purified rabbit polyclonal antibodies against leptin (Santa Cruz, Biotech, CA, USA) and goat polyclonal antibodies against OB-R (M-18, Santa Cruz Biotech)[30,32], respectively. Sections (3-μm thick) were deparaffinized in xylene, hydrated through a graded series of ethanol, and heated in a microwave oven for two 7-min cycles (500 W). After being rinsed in phosphate buffered saline (PBS), endogenous peroxidase activity was inhibited by incubation with 0.3% hydrogen peroxide in 100% methanol for 30 min. After 3 washes in PBS, nonspecific reaction was blocked by incubation with PBS containing 5% skimmed milk for 30 min at room temperature, and then the sections were incubated with normal rabbit or goat serum for 30 min. The sections were incubated overnight at 4℃ in humid chambers with the primary antibody to leptin at 1/70 dilution or the primary antibody to OB-R at 1/100. After three washes with PBS, the sections were incubated with biotinylated rabbit anti-goat or anti-rabbit immunoglobulin for 30 min. After washing again with PBS, the slides were treated with peroxidase-conjugated streptavidin for 30 min, and developed by immersion in 0.01% H_2O_2 and 0.05% diaminobenzidine tetrahydrochloride for 3 min. Light counterstaining with Mayer's hematoxylin was performed.

Statistical analysis

All statistical calculations were carried out using Stat View-J 5.0 statistical software (SAS Institute, USA). Student's t-test and Wilcoxon's test were used to analyze data. Differences with a P value of less than 0.05 were considered to be statistically significant.

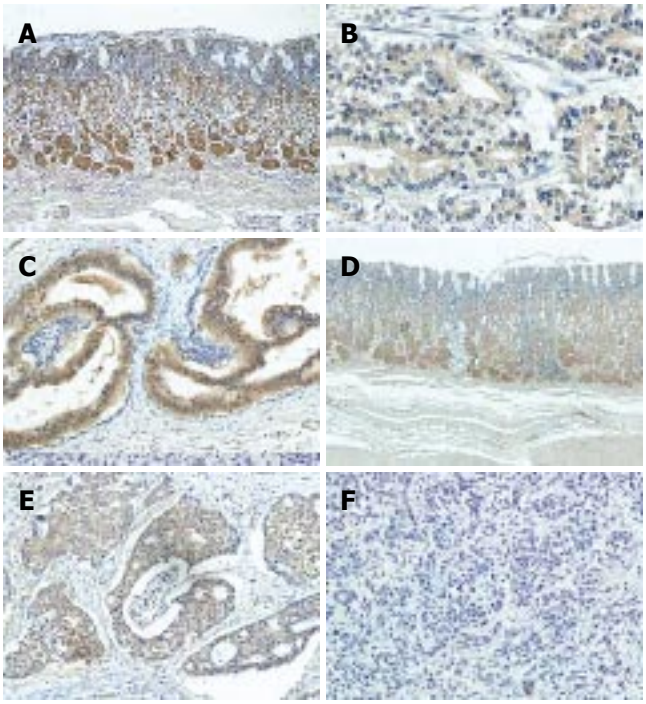


Figure 1 Immunohistochemical staining of leptin and OB-R in normal gastric mucosa and cancer. **A**: Leptin in normal mucosa; **B**: Leptin weak type in gastric cancer; **C**: leptin strong type in gastric cancer; **D**: OB-R in normal mucosa; **E**: OB-R positive type in gastric cancer; **F**: OB-R negative type in gastric cancer.

RESULTS

Immunohistochemical detection of leptin and OB-R in normal mucosa and carcinoma

In all cases, the lower part of the fundic glands in the normal part of the mucosa expressed a significant level of leptin, suggesting that leptin is mainly produced in chief and parietal cells (Figure 1A). Leptin could be detected in the cytoplasm as well as the cell membrane, but not in the nucleus. However, the surface epithelium of normal gastric mucosa totally lacked expression of leptin. This staining pattern was similar to that described in the gastric epithelium in a previous report[22].

Gastric carcinoma cells mostly showed positive immunoreactivity, although the staining intensity varied among the samples. According to the staining pattern, tumors were subdivided into two groups. When investigators agreed that the staining intensity of carcinoma cells was significantly weaker than that of chief and parietal cells in corresponding normal mucosa, those tumors were categorized as having weak expression (Figure 1B). In contrast, when carcinoma cells stained to a similar degree or more strongly than normal gastric mucosa, those tumors were categorized as having strong expression (Figure 1C).

OB-R was also detected in normal mucosa, and the immunostaining pattern was mostly consistent with that of leptin staining (Figure 1D). In cancer tissue, however, some carcinoma cells showed significant expression while

Table 1 Relationship between expression of Ob-R and leptin

	Ob-R expression		
	Positive (67)	Negative (140)	*P*
Leptin expression			
Strong (74)	45	29	
Weak (133)	22	111	< 0.001

others were mostly negative for OB-R. In tumors positive for OB-R, most of the carcinoma cells were equally stained and heterogeneity was rarely observed in each sample (Figure 1E), while in negative tumors a few carcinoma cells were stained only faintly (Figure 1F).

The relationship between the expression patterns of leptin and OB-R is presented in Table 1. Among 74 carcinomas with strong expression of leptin, 45 (60.8%) also expressed OB-R strongly, while only 29 carcinomas (39.2%) lacked expression of OB-R. In the 133 carcinomas with weak leptin expression, 111 (79.3%) also lacked expression of leptin OB-R. Hence, the expression of leptin and OB-R was significantly correlated in gastric cancer ($P < 0.001$).

Clinicopathological features and relation to expression of leptin and OB-R

Table 2 shows the correlation between leptin and OB-R expression and clinicopathological data in the 207 carcinomas cases. No significant difference was observed in age, preoperative tumor markers or tumor size between the positive and negative groups. Interestingly, positive expression of leptin was found in 64 of 155 male patients (41.3%) versus 10 of 52 female patients (19.2%), and the difference was statistically significant ($P < 0.05$). The relationship of BMI with leptin expression in the two groups did not show a significant association. The stomach is anatomically divided into three portions; upper (U), middle (M), lower (L) parts[33]. Interestingly, the percentage of tumors with strong expression of leptin was higher in those located in the upper part (23/51; 45.1%) than in those in the middle (36/94; 38.3%) and lower (15/62; 24.2%) parts, and this was statistically significant ($P < 0.05$).

The expression levels of both leptin and OB-R increased as the depth of tumor invasion or TMN stage increased ($P < 0.01$). When leptin expression was compared with histological type, 45 of 88 (51.1%) well differentiated carcinomas expressed leptin strongly, while 90 of 119 (75.6%) undifferentiated carcinomas showed weak staining ($P < 0.001$). Moreover, the percentage of tumors with OB-R-positive expression was significantly higher in differentiated carcinomas (48.9%) than in undifferentiated carcinomas (20.1%) ($P < 0.001$). Thus, both leptin and OB-R were expressed at a higher level in differentiated carcinomas as compared with undifferentiated carcinomas ($P < 0.001$).

Venous as well as lymphatic invasion was more frequently observed in tumors with high leptin and positive OB-R expression. Accordingly, lymph node metastasis was detected in 71.6% (48/67) of OB-R-positive cases, which was significantly higher than 31.3% (47/150) of OB-R-negative cases. Interestingly, in the 74 tumors with high leptin expression, hematogenous metastasis was detected preoperatively in 3 (4.1%) patients, and peritoneal dissemination was detected intraoperatively in 5 (6.8%) patients. However, in 133 tumors with low leptin expression, only one case showed peritoneal dissemination and none was associated with hematogenous metastasis ($P < 0.05$).

DISCUSSION

Leptin is well known to play a major role in the regulation of weight and adiposity. Recently, many studies have shown that increased body weight is associated with increased risk of cancer and cancer-related mortality, suggesting a possible role of leptin in the pathogenesis of cancer. Leptin is reported to be abundantly produced in the stomach[34,35]. In gastric carcinoma, some reports have shown that obesity is one of the main risk factors[36-42]. These findings suggest that leptin may be critically involved in the development and progression of gastric cancer. This idea encouraged us to evaluate the expression of leptin and its receptor in gastric cancer tissues.

In our series, leptin and OB-R were predominantly expressed in chief and parietal cells but not in the surface epithelium in normal parts of the gastric mucosa that were adjacent to cancer tissue, which is mostly consistent with data of previous studies[22,43,44]. However, carcinoma cells showed a variety of staining patterns for leptin or OB-R. Leptin was detected in all carcinoma cells, although the level of expression could be divided into two categories according to the staining intensity, whereas OB-R was detected in some tumors but not in others, and the level of expression of leptin showed a positive correlation with OB-R expression. This suggests the existence of a common regulatory mechanism in the expression of leptin and its receptor in the gastric epithelium.

The main finding in our study was that the expression levels of both leptin and OB-R tended to increase as the depth of tumor invasion or TMN stage increased ($P < 0.01$). Moreover, nodal and distant metastasis, as well as pathological lymphatic or vascular invasion, was frequently detected in leptin-strong and OB-R-positive tumors as compared with leptin-weak and OB-R-negative tumors. Shuneider *et al*[43] reported that leptin led to significantly increased proliferation in AGS cells, and the MAP-kinase-1 specific inhibitor U0126 blocked leptin-induced cell proliferation in a dose-dependent manner. Tessitore reported that in colorectal cancer patients, plasma leptin level in stage Ⅳ patients was significantly higher than that in stage I patients. In addition to stimulating proliferation, leptin has been shown to promote invasiveness of renal and colonic epithelial cells via PI3-kinase-, rho- and rac-dependent cascades[25]. All these findings support that leptin may have a promoting effect on cancer invasion and metastasis. Our findings were consistent with these results and suggest that leptin and OB-R might function as an autocrine growth factor during the development and progression of gastric cancer.

Another interesting finding was that the expression of

Table 2 Expression of leptin and clinicopathologic characteristics of patients

	Leptin expression			OB-R expression		
	High (74)	Low (133)	*P*	Positive (67)	Negative (140)	*P*
Age (yr)	62.7 ± 8.9	61.3 ± 11.2	0.98	64.2 ± 9.9	61.6 ± 10.8	0.167
Sex						
Male	64	91		53	102	
Female	10	42	0.003	14	38	0.33
BMI	22.4 ± 3.1	22.7 ± 2.8	0.48	22.4 ± 3.2	22.7 ± 2.8	0.49
Tumor markers						
CEA	8.6 ± 17.7	14.7 ± 111.3	0.65	9.9 ± 19.6	13.8 ± 108.3	0.79
CA19-9	89.8 ± 453.5	75.4 ± 347.6	0.81	138.3 ± 561.4	53.8 ± 270.8	0.16
Size (cm)	6.0 ± 3.2	5.1 ± 3.6	0.13	5.8 ± 3.2	5.2 ± 3.6	0.25
Location						
Upper part (51)	23	28		19	32	
Middle part (94)	36	58		29	65	
Lower part (62)	15	47	0.03	19	43	0.51
Depth of tumor invasion						
T1	24	76		20	80	
T2	28	29		27	37	
T3	18	28		16	30	
T4	4	0	0.001	4	0	< 0.001
Macroscopic type						
Elevated	57	62		48	71	
Depressed/flat	17	71	< 0.001	19	69	0.005
Histological type						
Differentiated	45	43		43	45	
Undifferentiated	29	90	< 0.001	24	95	< 0.001
TNM stage						
IA	20	67		15	72	
IB	17	23		15	25	
Ⅱ	8	12		9	11	
ⅢA	10	17		11	16	
ⅢB	5	7		7	5	
Ⅳ	14	7	0.001	10	11	< 0.001
Lymphatic invasion						
Positive	39	45		39	46	
Negative	34	87	0.002	28	94	< 0.001
Venous invasion						
Positive	46	49		45	50	
Negative	28	84	< 0.001	22	90	< 0.001
Lymph node metastasis						
Positive	47	48		48	47	
Negative	37	85	0.052	19	93	0.001
Hematogenous metastasis						
Positive	3	0		2	1	
Negative	71	134	0.02	65	139	0.19
Peritoneal dissemination						
Positive	5	1		2	4	
Negative	69	133	0.01	65	136	0.95

leptin/OB-R was correlated with the differentiation of gastric cancer. In our series, cancers of undifferentiated type tended to have weak expression of leptin as well as negative OB-R expression as compared with differentiated cancers. In each type, expression of leptin/OB-R showed a positive association with stage and metastasis (data not shown). This suggests that the different expression of leptin/OB-R was determined at the early stage of carcinogenesis. The carcinogenic pathway of differentiated type carcinoma is considered to begin with *H pylori* infection, followed by atrophic gastritis and intestinal metaplasia, and inappropriate activation of gut specific transcription factor CDX2 has an important role in the early stage of carcinogenesis. In contrast, dysfunction

of E-cadherin is considered to have critical roles in the development of undifferentiated carcinoma. The molecular interaction between leptin/OB-R and CDX2 or E-cadherin is an interesting subject for future research[45].

In conclusion, we confirmed that the expression level of leptin/OB-R showed a positive correlation with the depth of tumor invasion, stage, and metastasis as well as tumor differentiation. Our findings suggest that coexpression of leptin and OB-R may have a positive role in the progression in gastric cancer in an autocrine or paracrine manner. Functional inhibition of leptin/OB-R might effectively suppress the growth and metastasis of gastric cancer.

REFERENCES

1 **Fund WCR**. Food, nutrition and the prevention of cancer: a global perspective. American Institute for Cancer Research, 1997: 371-373
2 **Carroll KK**. Obesity as a risk factor for certain types of cancer. *Lipids* 1998; **33**: 1055-1059
3 **Bergström A**, Pisani P, Tenet V, Wolk A, Adami HO. Overweight as an avoidable cause of cancer in Europe. *Int J Cancer* 2001; **91**: 421-430
4 **Vainio H**, Kaaks R, Bianchini F. Weight control and physical activity in cancer prevention: international evaluation of the evidence. *Eur J Cancer Prev* 2002; **11** Suppl 2: S94-S100
5 **Peto J**. Cancer epidemiology in the last century and the next decade. *Nature* 2001; **411**: 390-395
6 **Correa P**. Epidemiological correlations between diet and cancer frequency. *Cancer Res* 1981; **41**: 3685-3690
7 **Carroll KK**. Neutral fats and cancer. *Cancer Res* 1981; **41**: 3695-3699
8 **Cleary MP**, Maihle NJ. The role of body mass index in the relative risk of developing premenopausal versus postmenopausal breast cancer. *Proc Soc Exp Biol Med* 1997; **216**: 28-43
9 **Willett WC**. Fat, energy and breast cancer. *J Nutr* 1997; **127**: 921S-923S
10 **Calle EE**, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003; **348**: 1625-1638
11 **Collins S**, Kuhn CM, Petro AE, Swick AG, Chrunyk BA, Surwit RS. Role of leptin in fat regulation. *Nature* 1996; **380**: 677
12 **Lindheim SR**, Sauer MV, Carmina E, Chang PL, Zimmerman R, Lobo RA. Circulating leptin levels during ovulation induction: relation to adiposity and ovarian morphology. *Fertil Steril* 2000; **73**: 493-498
13 **Henson MC**, Castracane VD. Leptin in pregnancy. *Biol Reprod* 2000; **63**: 1219-1228
14 **Zhang Y**, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; **372**: 425-432
15 **Caro JF**, Sinha MK, Kolaczynski JW, Zhang PL, Considine RV. Leptin: the tale of an obesity gene. *Diabetes* 1996; **45**: 1455-1462
16 **Caro JF**. Leptin: from 1958 to the present. Can J Diabetes Care, 1998: 18-23
17 **Flier JS**, Maratos-Flier E. Obesity and the hypothalamus: novel peptides for new pathways. *Cell* 1998; **92**: 437-440
18 **Dagogo-Jack S**, Fanelli C, Paramore D, Brothers J, Landt M. Plasma leptin and insulin relationships in obese and nonobese humans. *Diabetes* 1996; **45**: 695-698
19 **Smith FJ**, Rivera I, Tanenbaum R, et al. Weight loss reverses decreased sensitivity to exogenous ob protein (leptin) in diet induced obese mice. *Int J Obesity* 1998; 41
20 **Ganong WF**. Review of Medical Physiology. 18th ed. New York: Appleton and Lange, 1997: 223
21 **Hoggard N**, Hunter L, Duncan JS, Williams LM, Trayhurn P, Mercer JG. Leptin and leptin receptor mRNA and protein expression in the murine fetus and placenta. *Proc Natl Acad Sci USA* 1997; **94**: 11073-11078
22 **Mix H**, Widjaja A, Jandl O, Cornberg M, Kaul A, Göke M, Beil W, Kuske M, Brabant G, Manns MP, Wagner S. Expression of leptin and leptin receptor isoforms in the human stomach. *Gut* 2000; **47**: 481-486
23 **Isono M**, Inoue R, Kamida T, Kobayashi H, Matsuyama J. Significance of leptin expression in invasive potential of pituitary adenomas. *Clin Neurol Neurosurg* 2003; **105**: 111-116
24 **Briscoe CP**, Hanif S, Arch JR, Tadayyon M. Leptin receptor long-form signalling in a human liver cell line. *Cytokine* 2001; **14**: 225-229
25 **Attoub S**, Noe V, Pirola L, Bruyneel E, Chastre E, Mareel M, Wymann MP, Gespach C. Leptin promotes invasiveness of kidney and colonic epithelial cells via phosphoinositide 3-kinase-, rho-, and rac-dependent signaling pathways. *FASEB J* 2000; **14**: 2329-2338
26 **Cioffi JA**, Shafer AW, Zupancic TJ, Smith-Gbur J, Mikhail A, Platika D, Snodgrass HR. Novel B219/OB receptor isoforms: possible role of leptin in hematopoiesis and reproduction. *Nat Med* 1996; **2**: 585-589
27 **Emilsson V**, Liu YL, Cawthorne MA, Morton NM, Davenport M. Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. *Diabetes* 1997; **46**: 313-316
28 **Glasow A**, Haidan A, Hilbers U, Breidert M, Gillespie J, Scherbaum WA, Chrousos GP, Bornstein SR. Expression of Ob receptor in normal human adrenals: differential regulation of adrenocortical and adrenomedullary function by leptin. *J Clin Endocrinol Metab* 1998; **83**: 4459-4466
29 **Morash B**, Johnstone J, Leopold C, Li A, Murphy P, Ur E, Wilkinson M. The regulation of leptin gene expression in the C6 glioblastoma cell line. *Mol Cell Endocrinol* 2000; **165**: 97-105
30 **Tsuchiya T**, Shimizu H, Horie T, Mori M. Expression of leptin receptor in lung: leptin as a growth factor. *Eur J Pharmacol* 1999; **365**: 273-279
31 **Hino M**, Nakao T, Yamane T, Ohta K, Takubo T, Tatsumi N. Leptin receptor and leukemia. *Leuk Lymphoma* 2000; **36**: 457-461
32 **Hardwick JC**, Van Den Brink GR, Offerhaus GJ, Van Deventer SJ, Peppelenbosch MP. Leptin is a growth factor for colonic epithelial cells. *Gastroenterology* 2001; **121**: 79-90
33 **Japanese Gastric Cancer Association.** Japanese Classification of Gastric Carcinoma - 2nd English Edition. *Gastric Cancer* 1998; **1**: 10-24
34 **Bado A**, Levasseur S, Attoub S, Kermorgant S, Laigneau JP, Bortoluzzi MN, Moizo L, Lehy T, Guerre-Millo M, Le Marchand-Brustel Y, Lewin MJ. The stomach is a source of leptin. *Nature* 1998; **394**: 790-793
35 **Brzozowski T**, Konturek PC, Konturek SJ, Pajdo R, Duda A, Pierzchalski P, Bielański W, Hahn EG. Leptin in gastroprotection induced by cholecystokinin or by a meal. Role of vagal and sensory nerves and nitric oxide. *Eur J Pharmacol* 1999; **374**: 263-276
36 **Augustin LS**, Gallus S, Negri E, La Vecchia C. Glycemic index, glycemic load and risk of gastric cancer. *Ann Oncol* 2004; **15**: 581-584
37 **Chow WH**, Blot WJ, Vaughan TL, Risch HA, Gammon MD, Stanford JL, Dubrow R, Schoenberg JB, Mayne ST, Farrow DC, Ahsan H, West AB, Rotterdam H, Niwa S, Fraumeni JF Jr. Body mass index and risk of adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst* 1998; **90**: 150-155
38 **Lagergren J**, Bergström R, Nyrén O. Association between body mass and adenocarcinoma of the esophagus and gastric cardia. *Ann Intern Med* 1999; **130**: 883-890
39 **Bremner CG**, Lynch VP, Ellis FH Jr. Barrett's esophagus: congenital or acquired? An experimental study of esophageal mucosal regeneration in the dog. *Surgery* 1970; **68**: 209-216
40 **Vaughan TL**, Farrow DC, Hansten PD, Chow WH, Gammon MD, Risch HA, Stanford JL, Schoenberg JB, Mayne ST, Rotterdam H, Dubrow R, Ahsan H, West AB, Blot WJ, Fraumeni JF Jr. Risk of esophageal and gastric adenocarcinomas in relation to use of calcium channel blockers, asthma drugs, and other medications that promote gastroesophageal reflux. *Cancer Epi-*

demiol Biomarkers Prev 1998; **7**: 749-756

41 **You WC**, Blot WJ, Chang YS, Ershow AG, Yang ZT, An Q, Henderson B, Xu GW, Fraumeni JF Jr, Wang TG. Diet and high risk of stomach cancer in Shandong, China. *Cancer Res* 1988; **48**: 3518-3523

42 **Galanis DJ**, Kolonel LN, Lee J, Nomura A. Intakes of selected foods and beverages and the incidence of gastric cancer among the Japanese residents of Hawaii: a prospective study. *Int J Epidemiol* 1998; **27**: 173-180

43 **Schneider R**, Bornstein SR, Chrousos GP, Boxberger S, Ehninger G, Breidert M. Leptin mediates a proliferative response in human gastric mucosa cells with functional receptor. *Horm Metab Res* 2001; **33**: 1-6

44 **Azuma T**, Suto H, Ito Y, Ohtani M, Dojo M, Kuriyama M, Kato T. Gastric leptin and Helicobacter pylori infection. *Gut* 2001; **49**: 324-329

45 **Yuasa Y**. Control of gut differentiation and intestinal-type gastric carcinogenesis. *Nat Rev Cancer* 2003; **3**: 592-600

S- Editor Wang GP **L- Editor** Schreyer AG **E- Editor** Ma WH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5523-5527
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Luminal oxidants selectively modulate electrogenic ion transport in rat colon

Julio M Mayol, Yolanda Adame-Navarrete, Pilar Alarma-Estrany, Elena Molina-Roldan, Fernando Huete-Toral, Jesus A Fernandez-Represa

Julio M Mayol, Yolanda Adame-Navarrete, Pilar Alarma-Estrany, Elena Molina-Roldan, Fernando Huete-Toral, Jesus A Fernandez-Represa, Servicio De Cirugia I, Hospital Clinico San Carlos, Universidad Complutense De Madrid, Madrid 28040, Spain
Supported by grants from Ministerio de Ciencia y Tecnología (BFI 03/1350) and Fundacion MMA
Correspondence to: Julio M. Mayol, Servicio de Cirugia I (3ª N), Hospital Clinico San Carlos, Madrid 28040, Spain. jmayol@eresmas.net
Telephone: +34-91-3304167 Fax: +34-91-3303166
Received: 2006-04-30 Accepted: 2006-06-14

Abstract

AIM: To investigate the effects of luminal exposure to H_2O_2 and two related thiol oxidizing agents on basal and stimulated chloride secretion in native colon using electrophysiological and pharmacological approaches.

METHODS: Unstripped rat distal colon segments were mounted in Ussing chambers. Potential difference, calculated resistance and short-circuit current across unstripped colon segments were monitored with a dual voltage/current clamp. Paracellular permeability was assessed by measuring the mucosa-to-serosa flux of a fluorescent probe (FITC).

RESULTS: Luminal exposure to hydrogen peroxide transitorily stimulated chloride secretion without altering barrier function. This stimulatory effect could be blocked by basolateral atropine but not indomethacin. The cysteine and methionine oxidizing compounds, phenylarsine oxide and chloramine T respectively, mimicked the effect of H_2O_2, except for a drop in transcolonic resistance after 30 min. In contrast to the observed stimulatory effect on basal secretion, cAMP-stimulated electrogenic ion transport was blunted by luminal H_2O_2. However, the Ca^{2+}-activated response remained unchanged.

CONCLUSION: H_2O_2 may be an important selective modulator of intestinal ion and water secretion in certain pathologic conditions such as inflammation or ischemia-reperfusion by multiple mechanisms.

Key words: Chloride secretion; Ion transport; Rat distal colon; Hydrogen peroxide; Acetylcholine; Atropine

Mayol JM, Adame-Navarrete Y, Alarma-Estrany P, Molina-Roldan E, Huete-Toral F, Fernandez-Represa JA. Luminal oxidants selectively modulate electrogenic ion transport in rat colon. *World J Gastroenterol* 2006; 12(34): 5523-5527

http://www.wjgnet.com/1007-9327/12/5523.asp

INTRODUCTION

The colonic lumen is an extraordinarily aggressive milieu containing high concentrations of oxidizing compounds[1] as a result of the digestion of nutrients, the presence of dietary oxidants and the metabolic activity of resident colonic flora. Moreover, many gastrointestinal diseases, such as intestinal ischemia, ischemic colitis or inflammatory bowel disease, are associated with an increase in the concentration of both reactive oxygen and nitrogen species in the intestinal mucosa[2], generated by either activated neutrophils infiltrating the colonic crypts or epithelial cells submitted to ischemia/reperfusion. Therefore, colonic epithelial cells and submucosal tissue are under continuous luminal oxidative stress in states of health and disease, but little is known about the impact of luminal oxidants on mucosal functions in intact native mammalian colon since most studies have been carried out in cell lines.

Oxidants have been shown to interfere with epithelial ion transport and barrier function. In intestinal model cell lines, addition of hydrogen peroxide, either basolaterally or apically, first activates vectorial Cl^- transport and then inhibits cAMP-activated anion secretion. The inhibitory effect on the forskolin-stimulated response appears to be the result of the blockade of apical Cl^- conductance and the inhibition of Na^+-K^+-ATPase activity[3]. In animal models, serosal application of H_2O_2, at micromolar concentrations, to muscle-stripped rat colon evokes a transient increase in anion secretion[4]. Yet, the role of luminal oxidants on stimulated transport in intact native colon remains poorly understood. Therefore, our aim was to investigate the effect of luminal H_2O_2 on basal and stimulated electrogenic ion transport in rat distal colon.

MATERIALS AND METHODS

Rat distal colon model in vitro

Male Sprague-Dawley rats weighing 250-350 g were killed by intraperitoneal administration of sodium pentobarbital.

Animals were handled in accordance with the protocols approved by Comisión de Investigación-Hospital Clínico San Carlos, in compliance with both Spain's and European Union's regulations for the use of animals in biomedical research.

The method to obtain samples for electrophysiological studies has been reported elsewhere[5,6]. In brief, a 5-cm segment of distal colon was surgically removed and placed in iced buffer solution. The specimen was opened along its mesenteric side. Unstripped colonic segments were mounted in modified chambers (Costar, San Diego CA) with a surface area of 0.64 cm^2. These chambers could provide individualized access to both the luminal and the serosal compartments of the colon. Both chambers were bathed with an identical volume (5 mL) of the buffer solution containing 122.0 mmol/L NaCl, 2.0 mmol/L $CaCl_2$, 1.3 mmol/L $MgSO_4$, 5.0 mmol/L KCl, 20.0 mmol/L glucose and 25.0 mmol/L $NaHCO_3$ (pH 7.4 when gassed with 950 mL/L de O_2 and 50 mL/L CO_2 at 37℃).

Electrophysiology

Two Ag/AgCl electrodes connected to a dual voltage-current clamp (World Precision Instruments ECV 4000, Sarasota-FL, USA) were placed in the apical and basolateral chambers. Spontaneous transepithelial potential difference (E0; lumen negative, in mV) could therefore be monitored. Potential difference values were corrected for the junction potentials (< 0.1 mV) between the luminal and the serosal solutions. Two additional electrodes were used to apply a 50 μA current through the mounted colon. The resulting potential difference was measured (E50). Transcolonic resistance (TR, in $\Omega \cdot cm^2$) was calculated with E0 and E50 values by Ohm's law, reflecting epithelial viability and intestinal barrier function. Short circuit current (Isc, in $\mu A/cm^2$) could be obtained with E0 and TR values by Ohm´s law, indicating the amount of electrical current needed to nullify the spontaneous potential difference between the apical and the basolateral surfaces.

Electrophysiological experiments were carried out after the samples were mounted in the modified chambers and bathed in buffer solution until stable electrical activity was reached. The response of rat colon exposed to luminal oxidants to the muscarinic blocker (atropine) was studied after it was added to the basolateral chamber in the continued presence of both compounds. Similarly, the effect of two different secretagogues (forskolin and carbachol) on ion transport was assessed following the same protocol (luminal incubation + continued presence of the oxidant).

Paracellular permeability

Paracellular permeability was assessed by measuring the mucosal-to-serosal flux of fluorescein isothiocyanate (FITC; MW: 376.3) as previously described[6]. Paired rat distal colon segments were mounted in chambers and bilaterally incubated in regular buffer for 10 min for equilibration. Subsequently, the apical buffer was replaced with a FITC-containing solution (140 μmol/L) with or without hydrogen peroxide. Basolateral buffer aliquots were collected at 0, 5, 15, 30 and 60 min after apical buffer replacement and the fluorescent emission at 520 nm after excitation at 480 nm was measured with a spectrofluometer (Bio-Tek FL600 Fluorescence Microplate Reader, Bio-Tek Instruments GmbH, Bad Friedrichshall, Germany). A calibration curve (fluorescence *vs* FITC concentration) was generated to calculate the concentration of FITC in the serosal chamber. The apparent permeability coefficient (Papp) was calculated according to the equation $P_{app}\ (cm/seg) = (ct2/ct1) \cdot V \cdot A^{-1} \cdot C_0^{-1}$, where $(ct2/ct1)$ is the net increase in FITC concentration in the serosal buffer in a given interval of time (seconds), V is the volume (in milliliters) of the basolateral compartment, A is the surface of the colon segment (in square centimeters) and C_0 is the concentration in the apical chamber.

Materials

Cyclic AMP and Ca^{2+}-dependent secretion was stimulated with forskolin (10 μmol/L) and carbachol (100 μmol/L), respectively. Phenylarsine oxide (PAO, 0.2 mmol/L) and chloramine T (ClT, 5 mmol/L) were used as non-physiologic thiol oxidants. For muscarinic receptor inhibition, atropine at a concentration of 1 mg/L was employed. Indomethacin (1-10 μmol/L) was used for blockage of cyclo-oxygenase activity. Forskolin and indomethacin were added to both chambers (luminal and serosal). However, exposition to the muscarinic agonist and its antagonist was only from the serosal side. All the compounds were obtained from Sigma, Madrid-Spain.

Statistical analysis

Results were presented as mean ± SD. Student's *t* and repeated measure ANOVA tests were used for statistical comparisons when indicated. $P < 0.05$ was considered statistically significant.

RESULTS

Luminal exposure to H_2O_2 (up to 10 mmol/L) for 30 min did not alter either transcolonic resistance (TR of H_2O_2-treated samples = 92.2% ± 11.7% of controls; $n = 6$ for each group; NS) or paracellular permeability (Papp = $1.24 \times 10^{-6} \pm 0.16 \times 10^{-6}$ for control *vs* $0.95 \times 10^{-6} \pm 0.35 \times 10^{-6}$ for 10 mmol/L H_2O_2; $n = 6$ for each group, NS). However, as shown in Figure 1, a transient increase in Isc occurred 5 min after the addition of the oxidant to the apical chamber (peak Isc = 46.7 ± 2.8 $\mu A/cm^2$ for control samples, $n = 5$; *vs* 85.9 ± 9.6 $\mu A/cm^2$ for 10 mmol/L H_2O_2; $n = 6$, $P < 0.01$). This secretory response was dose-dependent (from 100 μmol/L to 10 mmol/L) and peaked at a concentration of ~8 mmol/L (ΔIsc = 32.3 ± 3.4 $\mu A/cm^2$ at 8 mmol/L; $n = 4$ for all groups; $P < 0.05$ for the comparison between controls and oxidant-treated samples). However, it was not specific for H_2O_2 since two widely used cysteine and methionine oxidizing agents, phenylarsine and chloramine T, at concentrations used by other investigators to study epithelial barrier function in cell lines (0.2 mmol/L and 5 mmol/L respectively), generated the same secretory response after 5 min but induced a slight drop in transcolonic resistance 30 min after addition (Table 1).

In order to investigate the implication of different

Table 1 Transcolonic resistance and maximal short circuit current in rat distal colon exposed to phenylarsine oxide (PAO, 0.2 mmol/L) and chloramine T (CIT, 5 mmol/L) for 30 min

Group	*n*	TR[1] at 30 min	peak Isc[2]
Control	8	113 ± 8.0	66.4 ± 6.8
PAO (0.2 mmol/L)	4	77 ± 14.0[a]	131.6 ± 16.7[a]
CIT (5 mmol/L)	4	66.5 ± 4.3[a]	166.6 ± 27.8[a]

[a]$P < 0.05$ *vs* control; [1]Transcolonic resistance; [2]Short circuit current.

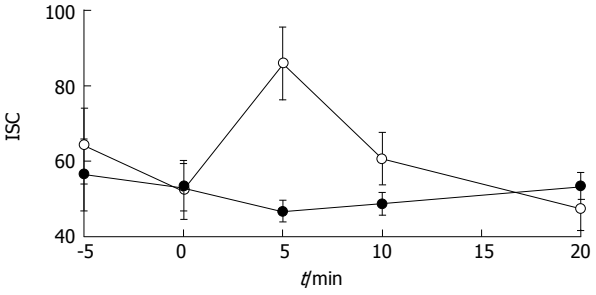


Figure 1 Luminal H_2O_2 transiently stimulates Cl secretion. After the addition of 10 mmol/L H_2O_2 to the apical surface of unstripped rat distal colon segments at time 0 (in white circles, $n = 4$), basal Isc increased significantly compared to control (black circles, $n = 4$), with maximal secretion occurring 5 min after the addition of the oxidant ($P < 0.001$).

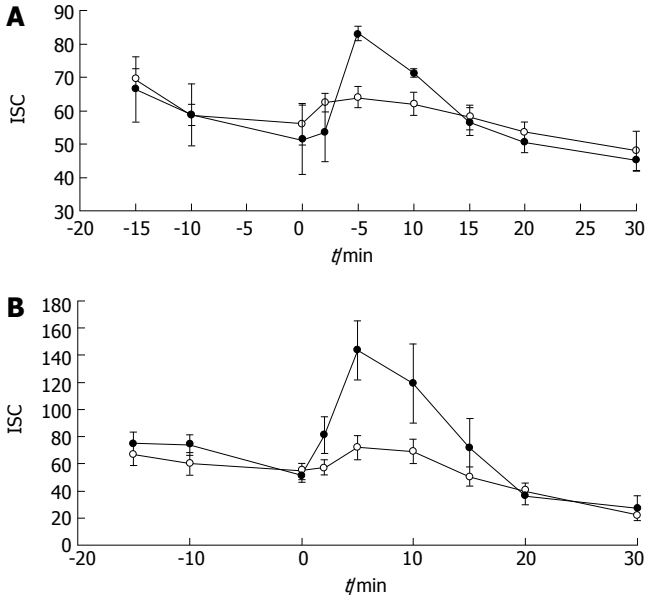


Figure 2 Oxidant-stimulated secretion is sensitive to basolateral atropine. Both phenylarsine oxide (PAO, 0.2 mmol/L) and chloramine T (CIT, 5 mmol/L) showed a similar prosecretory effect when they were added luminally to rat distal colon. **A**: Preincubation of colon segments with basolateral atropine (1 g/L) for ten minutes (added at time -10; in white circles, $n = 3$) suppressed the increase in Isc induced by luminal cysteine oxidizing agent PAO added at time 0 (control in black circles; $n = 3$; $P < 0.01$ between groups); **B**: A similar result was observed with the methionine oxidizing compound chloramine T. Basolateral incubation with 1 g/L of atropine (added at time -10; in white circles, $n = 8$) suppressed the secretory response caused by CIT added at time 0 (in black circles, $n = 8$; $P < 0.001$ between groups).

secretagogues in H_2O_2-induced secretion, colonic samples were first incubated with varying concentrations of indomethacin (1-10 μmol/L) and subsequently exposed to the three oxidizing compounds. No inhibition of the Isc rise caused by 10 mmol/L H_2O_2 was observed. For example, samples incubated with 2 μmol/L indomethacin after addition of 10 mmol/L H_2O_2 yielded a peak Isc of 110 ± 13.7 μA/cm^2, whereas for controls the peak Isc was 134 ± 14.2 μA/cm^2 ($n = 9$ for each group; NS). The same result was observed with the other two oxidants (data not shown). Since other mediators, such as acetylcholine, might be involved in the observed effect, the response to luminal oxidants after muscarinic receptor blockade was investigated. Interestingly, the secretory response elicited by the three compounds was suppressed by basolateral preincubation with atropine (1 g/L). The ΔIsc in atropine-treated samples after exposure to 10 mmol/L H_2O_2 was -1.3 ± 8.6 μA/cm^2, whereas in samples incubated in regular buffer the ΔIsc in response to the same concentration of H_2O_2 was 55.2 ± 13.7 μA/cm^2 ($n = 6$, $P < 0.01$). Both PAO and chloramine T displayed a similar behaviour (Figures 2A and B).

Subsequently, the effect of luminal H_2O_2 on stimulated secretion was studied. After 30 min of incubation with 10 mmol/L H_2O_2, when no drop in permeability occurred (TR after incubation for 30 min = 116.6 ± 15.8 Ω·cm^2 H_2O_2-treated samples *vs* 87.8 ± 19.7 Ω·cm^2 for control; $n = 6$ and $n = 5$ respectively; NS), forskolin stimulated-secretion was inhibited (Figure 3) (maximal Isc = 69.3 ± 9.4 μA/cm^2 for H_2O_2-treated samples *vs* 134.1 ± 23.1 μA/cm^2 for control ($n = 6$ and $n = 5$ respectively; $P < 0.01$). This inhibitory action was dose-dependent with an IC50-0.7 mmol/L. Since Ca^{2+}-dependent agonists could activate distinct signaling pathways and membrane transporters to generate electrogenic ion transport, we studied the effect of 10 mmol/L H_2O_2 on the carbachol-stimulated secretory response. In this case, samples exposed to the oxidant luminally or to regular buffer responded to the Ca^{2+} agonist in the same fashion (Figure 4).

DISCUSSION

Even under normal conditions, colonic epithelial cells are continuously exposed to luminal oxidants from different sources[7,8]. Since these compounds may interfere with epithelial functions, further understanding of the specific effects of reactive oxygen species on ion transport is necessary to design therapeutic strategies to prevent cell dysfunctions, such as hypersecretion and energy depletion, during inflammation or ischemia[1,2] . In this study, we showed that luminal hydrogen peroxide, within a physiologically relevant concentration range, could transitorily activate chloride secretion without altering either electrical resistance or paracellular permeability in rat distal colon. This effect might be due to either direct stimulation of membrane transport proteins or due to the activation of intracellular second messengers in epithelial cells. In fact, mucosal and serosal addition of H_2O_2 could potentiate Cl^- secretion in a synergistic fashion[9] in T84 cells previously stimulated with cAMP-agonists, suggesting that the Ca^{2+} signaling pathway might be involved in H_2O_2-triggered secretory response.

However, the maximal Isc induced by H_2O_2 in the T84

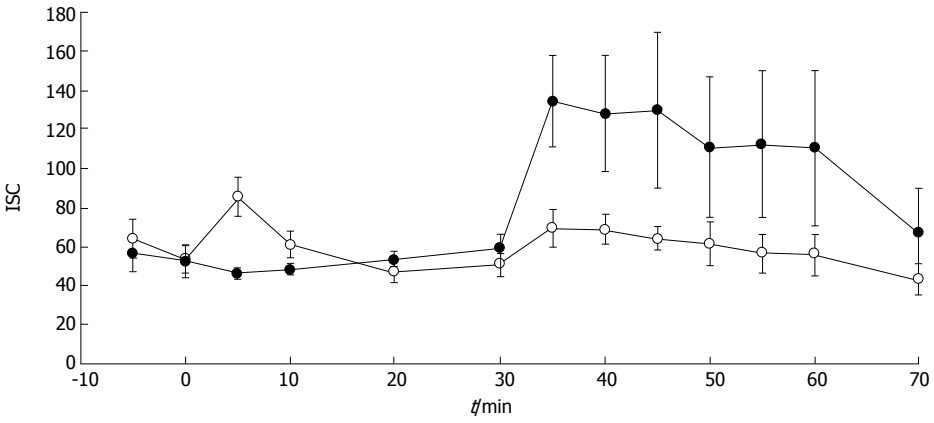


Figure 3 cAMP-activated secretion is blocked by luminal H_2O_2. Incubation of rat distal colon with 10 mmol/L H_2O_2 for 30 min (white circles; n = 6) inhibited the rise in Isc caused by 10 μmol/L forskolin added at time 0 (control in black circles; n = 6; P < 0.01) in white circles, n = 8) and suppressed the secretory response caused by CIT added at time 0 (in black circles, n = 8; P < 0.001 between groups).

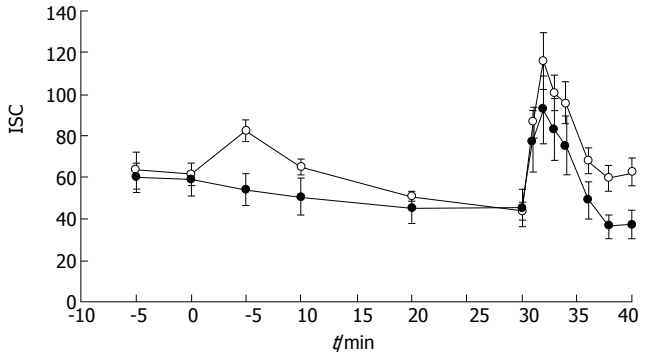


Figure 4 Ca^{2+}-stimulated Cl^- secretion is not affected by luminal by luminal H_2O_2. Incubation of rat distal colon with 10 mmol/L H_2O_2 for 30 min (white circles; n = 6) slightly enhanced the response induced by 100 μmol/L carbachol (control in black circles; n = 6; NS).

cell line is rather small compared to that observed in our model. Sugi *et al*[10] suggested that oxidants might enhance the response to secretagogues by priming of transport proteins in epithelial cell membrane. Interestingly, our results suggest that sudden increases in the concentration of luminal oxidants could activate secretion by releasing acetylcholine rather than by directly stimulating epithelial cells, which is independent from the generation of prostaglandins. This assertion is supported by the fact that the rise in Isc induced by three different oxidants-hydrogen peroxide, phenylarsine oxide and chloramine T, is almost completely suppressed by incubation with basolateral atropine but not with indomethacin at several concentrations. These findings are in contrast with the observations reported by Karayalcin *et al*[11], who found that basolateral hydrogen peroxide stimulates indomethacin-inhibitable secretion in stripped rat colon, which can be only partially blocked by atropine. Likewise, Tamai *et al*[4] showed that H_2O_2 increases Isc probably by stimulating release of arachidonate metabolites and neurotransmitters. This divergent finding may be due, at least in part, to the different "*in vitro*" models used. We studied the unstripped rat distal colon[12,13] because it could more accurately reflect the native tissue "*in vivo*" than the mucosa-submucosa preparations. Furthermore, Tamai *et al*[4] and Karayalcin *et al*[11] have used lower H_2O_2 concentrations and applied the oxidant to the serosal aspect of the stripped colonic wall.

An alternate explanation would imply that luminal H_2O_2 exerts a distinct action on each signaling pathway. That is, it might be priming the Ca^{2+}-dependent pathway while blocking the cAMP-dependent pathway. Thus, the stimulatory action of prostaglandins on colonic epithelial cells would be abolished and therefore, would become "invisible". To further support this hypothesis, previous studies in T84 cells have shown that incubation with H_2O_2 blocks chloride secretion stimulated by the cAMP agonist forskolin[3]. Similarly, we found that H_2O_2 could inhibit the cAMP-dependent secretory response, but not the activation of electrogenic ion transport by carbachol. In fact, the response to the Ca^{2+} agonist was slightly augmented. This finding points at a cyclic nucleotide-dependent membrane transport protein as the target for luminal oxidants. From previous studies, one can speculate that apical cAMP-dependent Cl^- channels are the most likely sites of action for these compounds[3,14]. The Na^+-K^+-ATPase is another potential target for oxidazing agents[3]. However, the discrepancy between the observed inhibition of the cAMP-dependent increase in vectorial anion transport and the absence of such an action on Ca^{2+}-stimulated secretion renders this explanation unlikely.

In conclusion, our results suggest that H_2O_2 may be an important selective modulator of intestinal ion and water secretion in certain pathologic conditions such as inflammation or ischemia-reperfusion by multiple mechanisms. Irrespective of the underlying mechanism of activation, the fact that an increase in the luminal concentration of reactive oxygen species triggers chloride secretion may be teleologically explained as a defensive mechanism for flushing microorganisms and toxic byproducts of bacterial metabolism away from the colonic lumen to dilute their concentration.

REFERENCES

1 **Halliwell B**, Zhao K, Whiteman M. The gastrointestinal tract: a major site of antioxidant action? *Free Radic Res* 2000; **33**: 819-830

2 **Jourd'heuil D**, Morise Z, Conner EM, Grisham MB. Oxidants, transcription factors, and intestinal inflammation. *J Clin Gastroenterol* 1997; **25** Suppl 1: S61-S72

3 **DuVall MD**, Guo Y, Matalon S. Hydrogen peroxide inhibits cAMP-induced Cl- secretion across colonic epithelial cells. *Am J Physiol* 1998; **275**: C1313-C1322

4 **Tamai H**, Kachur JF, Baron DA, Grisham MB, Gaginella TS. Monochloramine, a neutrophil-derived oxidant, stimulates rat

colonic secretion. *J Pharmacol Exp Ther* 1991; **257**: 887-894
5 **Mayol JM**, Alarma-Estrany P, O'Brien TC, Song JC, Prasad M, Adame-Navarrete Y, Fernández-Represa JA, Mun EC, Matthews JB. Electrogenic ion transport in mammalian colon involves an ammonia-sensitive apical membrane K+ conductance. *Dig Dis Sci* 2003; **48**: 116-125
6 **Mayol JM**, Alarma-Estrany P, Adame-Navarrete Y, Roldan EM, Toral FH, Fernandez-Represa JA. Effects of luminal ATPase inhibitors on electrogenic ion transport in rat distal colon. *J Surg Res* 2005; **129**: 85-89
7 **Erhardt JG**, Lim SS, Bode JC, Bode C. A diet rich in fat and poor in dietary fiber increases the in vitro formation of reactive oxygen species in human feces. *J Nutr* 1997; **127**: 706-709
8 **Nathan CF**. Neutrophil activation on biological surfaces. Massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. *J Clin Invest* 1987; **80**: 1550-1560
9 **Nguyen TD**, Canada AT. Modulation of human colonic T_{84} cell secretion by hydrogen peroxide. *Biochem Pharmacol* 1994; **47**: 403-410
10 **Sugi K**, Musch MW, Di A, Nelson DJ, Chang EB. Oxidants potentiate Ca(2+)- and cAMP-stimulated Cl(-) secretion in intestinal epithelial T84 cells. *Gastroenterology* 2001; **120**: 89-98
11 **Karayalcin SS**, Sturbaum CW, Wachsman JT, Cha JH, Powell DW. Hydrogen peroxide stimulates rat colonic prostaglandin production and alters electrolyte transport. *J Clin Invest* 1990; **86**: 60-68
12 **Stoner MC**, Scherr AM, Lee JA, Wolfe LG, Kellum JM. Nitric oxide is a neurotransmitter in the chloride secretory response to serotonin in rat colon. *Surgery* 2000; **128**: 240-245
13 **Prasad M**, Ito S, Silen W. Functional studies of in vitro rat distal colon before and after restitution. *Surgery* 1997; **121**: 430-439
14 **Jeulin C**, Fournier J, Marano F, Dazy AC. Effects of hydroxyl radicals on outwardly rectifying chloride channels in a cultured human bronchial cell line (16HBE14o-). *Pflugers Arch* 2000; **439**: 331-338

S- Editor Pan BR **L- Editor** Wang XL **E- Editor** Bi L

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5528-5531
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Timing of laparoscopic cholecystectomy for acute cholecystitis: A prospective non randomized study

George Tzovaras, Dimitris Zacharoulis, Paraskevi Liakou, Theodoros Theodoropoulos, George Paroutoglou, Constantine Hatzitheofilou

George Tzovaras, Dimitris Zacharoulis, Paraskevi Liakou, Theodoros Theodoropoulos, Constantine Hatzitheofilou, Department of Surgery, University of Thessaly School of Medicine, University Hospital of Larissa, Larissa 41335, Greece
George Paroutoglou, Department of Gastroenterology, University Hospital of Larissa, Larissa 41335, Greece
Correspondence to: George Tzovaras, MD, Assistant Professor of Surgery, 19 Agorogianni A. Street, Larissa 41335, Greece. gtzovaras@hotmail.com
Telephone: +30-2410-682730 Fax: +30-2410-611097
Received: 2006-05-13 Accepted: 2006-07-07

Abstract

AIM: To study the timing of laparoscopic cholecystectomy for patients with acute cholecystitis.

METHODS: Between January 2002 and December 2005, all American Society of Anesthesiologists classification (ASA) Ⅰ, Ⅱ and Ⅲ patients with acute cholecystitis were treated laparoscopically during the urgent (index) admission. The patients were divided into three groups according to the timing of surgery: (1) within the first 3 d, (2) between 4 and 7 d and (3) beyond 7 d from the onset of symptoms. The impact of timing on the conversion rate, morbidity and postoperative hospital stay was studied.

RESULTS: One hundred and twenty-nine patients underwent laparoscopic cholecystectomy for acute cholecystitis during the index admission. Thirty six were assigned to group 1, 58 to group 2, and 35 to group 3. The conversion rate and morbidity for the whole cohort of patients were 4.6% and 10.8%, respectively. There was no significant difference in the conversion rate, morbidity and postoperative hospital stay between the three groups.

CONCLUSION: Laparoscopic cholecystectomy for acute cholecystitis during the index admission is safe, regardless of the time elapsed from the onset of symptoms. This policy can result in an overall shorter hospitalization.

Key words: Acute cholecystitis; Laparoscopic cholecystectomy; Timing

Tzovaras G, Zacharoulis D, Liakou P, Theodoropoulos T, Paroutoglou G, Hatzitheofilou C. Timing of laparoscopic cholecystectomy for acute cholecystitis: A prospective non randomized study. *World J Gastroenterol* 2006; 12(34): 5528-5531

http://www.wjgnet.com/1007-9327/12/5528.asp

INTRODUCTION

Laparoscopic cholecystectomy (LC) has been established as the treatment of choice for the management of acute cholecystitis (AC), despite initial reservations, regarding the impact of this policy on the conversion rate and morbidity[1]. Several prospective randomized trials[2-4] suggest the superiority of early (within 72 h) over the delayed (after a few weeks interval) intervention. This 72 h limit, however, is difficult to be kept in many cases for a variety of reasons, referring to both patients and physicians. On the other hand, there is a paucity of solid data as to what happens in the period after this 72 h time frame. The speculation of a worse outcome, when attempting LC for AC during the urgent admission beyond this very early phase, is experience rather than evidence-based.

In our daily practice, we have realized that only a small number of patients with AC are managed surgically within this "gold window" of 72 h from the onset of symptoms. If the remaining majority of patients with AC are managed conservatively with interval cholecystectomy to follow, then an increased total hospitalization and subsequently increased cost can be expected. Furthermore, the subgroup of patients who do not respond to conservative treatment, as well as those who relapse while awaiting an interval cholecystectomy should be considered[5]. For these reasons, we have adopted a policy of performing a LC during the initial emergency/urgent admission for "all comers" with AC, regardless of time delay between its onset of symptoms and surgery.

In view of this policy, we examined prospectively the impact of the duration of symptoms on mortality, morbidity, conversion rate and postoperative hospital stay in patients who underwent LC for AC during the urgent (index) admission.

MATERIALS AND METHODS

Subjects

Between January 2002 and December 2005, all American Society of Anesthesiologists classification (ASA) Ⅰ, Ⅱ and Ⅲ patients admitted or referred to our unit with AC under the care of one consultant surgeon (GT) with a special interest in HPB and laparoscopic surgery, were treated with LC during the index admission, regardless of the time elapsed from the onset of symptoms. ASA IV patients were usually offered ultrasound-guided percutaneous cholecystectomy and therefore, were excluded from the study. Patients were considered having AC when they had five out of the following six positive criteria: persistent right upper quadrant pain, temperature > 37.5℃, WBC > 10×10^9/L, positive Murphy's sign, presence of gallstones on ultrasound in combination with wall thickening and/or fluid at the gallbladder fossa. The diagnosis of AC was confirmed by intraoperative findings and pathologic specimens. Patients with strong evidence of concomitant common bile duct (CBD) stones were not excluded from the study, but were treated initially with preoperative endoscopic retrograde cholangiopancreatograpgy (ERCP), sphincterotomy and CBD clearance, followed by LC after an interval of at least 24 h, in order to assure that no ERCP-related complication occurred. Patients with suspicion of CBD stones had preoperative MRCP, and if stones were detected, they were treated as above. Intraoperative cholangiogram was not performed in any of the cases. There were no other selection criteria and every effort was made to operate on all the patients as soon as theatre time was available, provided that any concomitant medical problem was previously dealt with. The latter resulted sometimes in what is called in the literature "physician delay"[6]. Laparoscopic cholecystectomy was attempted in all cases under general anesthesia. The usual four-trocar technique was used (10 mm umbilical, 10 mm subxiphoid, 5 mm subcostal midclavicular line, 5 mm anterior axillary line) but additional trocar was used as necessary. The gallbladder was aspirated in most of the cases in order to be grasped, and dissection of the Calot's triangle structures was always performed close to the gallbladder wall. Retrograde dissection was only exceptionally performed, when in doubt about the triangle's structures after the initial dissection.

Methods

The patients were divided in three groups according to the time between onset of symptoms and operation: (1) within 3 d (early group), (2) between 4 to 7 d (intermediate group) and (3) ⩾ 8 d (delayed group). All data including demographics, preoperative, operative findings and postoperative information were collected prospectively into a computerized database. The episode of AC was considered simple (oedematous, hydrops) or complicated (empyema, gangrenous, emphysematous, concomitant choledocholithiasis or pancreatitis). The aim of the study was to detect the impact of the time elapsed from onset of symptoms to operation on the conversion rate, 30-d mortality, 30-d morbidity with special attention to bile duct injury incidence and length of postoperative hospital stay.

Table 1 Impact of delay in laparoscopic cholecystectomy on outcomes

Outcome	Ⅰ: ⩽ 3 d (*n* = 36)	Ⅱ: 4-7 d (*n* = 58)	Ⅲ: ⩾ 8 d (*n* = 35)	*P*
Conversion rate	1 (2.8%)	2 (3.4%)	3 (8.5%)	NS
Mortality	0	0	0	NS
Morbidity	3 (8.3%)	6 (10.3%)	5 (14.2%)	NS
Postop hospital stay	2 (1-6) d	2 (1-14) d	2 (1-35) d	NS

NS: no significant difference.

Statistical analysis

Statistical analysis was performed using the Arcus Quickstat biomedical statistical package (Research Solutions, UK) with the median values for continuous variables presented with range in parentheses. Fisher's exact test and Mann Whitney U test were used as appropriate to compare the groups to each other. $P < 0.05$ (two-tailed test) was considered statistically significant.

RESULTS

One hundred and twenty-nine patients underwent LC for AC during the index admission according to the protocol. During the same period some 453 elective laparoscopic cholecystectomies were performed by the same team. Thirty-six of the patients with acute cholecystitis (28%) had their operation within the first 3 d from the onset of their illness, 58 patients (45%) between 4 to 7 d and the other 35 patients (27%) after the first week. None of our patients had a more than 48 h delay due to unavailability of theatre space. Any other delay from the onset of symptoms to operation was attributed to either patients' delayed presentation/referral to our unit or to concomitant medical problems needing to be addressed preoperatively. Special mention should be made of a subgroup of patients with AC whose surgery was delayed due to the intake of anti-coagulants or more often anti-platelet agents, due to the dramatically increased use of these drugs during the last decade.

The impact of timing of LC on outcomes is shown in Table 1. Although the conversion rate was somewhat higher in the "delayed" group, this difference was not significant when this group was compared to either the "early" ($P = 0.35$, Fisher's exact test) or the "intermediate" group ($P = 0.36$, Fisher's exact test). Similarly, there was no significant difference in mortality, morbidity and postoperative hospital stay between the three groups of patients. Interestingly, this was noted despite the fact that a significantly higher number of complicated cases of AC were found among patients of the "intermediate" group, compared to those who underwent earlier operations. This was also reflected on the operative time difference between the groups (Table 2). No major bile duct injuries occurred. Four cases had bile leak, two in the "intermediate" group and two in the "delayed" group. The first was attributed to the gallbladder fossa, the second from an avulsed cystic duct and the remaining two from a friable cystic stump.

The first case eased spontaneously after 48 h, the other three cases with bile leak were treated successfully with ERCP, sphincterotomy and stenting of the common bile duct. Other complications and their treatment are shown in Table 3.

DISCUSSION

Acute cholecystitis which is generally found in approximately 20% of all admissions for gallstone disease[7] is no longer considered a contraindication for laparsoscopic cholecystectomy. In fact, urgent LC is now considered the optimal treatment of patients with AC[1]. Early LC has been proven superior to delayed interval LC in most of the prospective randomized trials. It results in a shorter hospital stay and a shorter recuperation time while the conversion rate and morbidity remain similar with or even lower than delayed interval LC[2-4]. How early is "early" is not clear in the literature, as this parameter has not been effectively tested in controlled randomized trials. All these prospective randomized trials comparing early and delayed interval LC, refer to the first 48-72 h for the early group, making this group somewhat highly selected. In daily practice very few patients are able to have surgical treatment during this short period of time, due to either patient or/and physician delay[6]. Very often patients present with delay or they are referred with delay by their physicians. Others suffer from co-morbidities needing consultation with other specialties preoperatively, while some require other intervention preoperatively, i.e. ERCP. A significant number of patients take oral anti-coagulants or anti-platelet agents requiring reversing before surgery. For all these reasons many patients in reality cannot have surgery within this time frame. In the present series some 72% of patients with acute cholecystitis were treated surgically during the index admission beyond the 72 h boundary, which is not very different from the reported experience by other authors[8-10]. There were no solid data regarding the optimal policy for this large group of patients treated outside this 72 h boundary. To our knowledge, there is only one small prospective randomized trial designed to address this issue[11]. Chandler *et al*[11] found that there is no difference in the conversion rate or morbidity between the early group (*n*: 21, surgery as soon as theatre schedule allowed) and the delayed group (*n*: 22, surgery during the index admission, after resolution of symptoms or failure to resolve on 5 d course of conservative treatment). Results from other comparative non-randomized trials of early and delayed LC during the urgent admission for AC are rather conflicting and most of these however indicate a higher conversion rate for the delayed group, but no difference in morbidity[6,8,9,12-14]. The definition of the so called "early" group among trials is also confusing. Some trials define early group counting from the time of admission or diagnosis rather than the time of onset of symptoms. This could be sometimes misleading, as the onset time of episode could differ significantly from the time of admission. We believe that counting from the onset of symptoms is more representative of the reality. Furthermore, all the studies were designed by using a boundary either of 48, 72 or 96 h from either onset of symptoms or time of admission, in order to compare the two groups of population. They included invariably patients who had surgery within the first 7 d for comparison. In our study, the patients were divided into three groups, including those who were treated surgically during the index admission even beyond one week from the onset of their illness.

Table 2 Demographic and perioperative characteristics of patients with acute cholecystitis

Characteristic	Ⅰ: ⩽ 3 d (*n* = 36)	Ⅱ: 4-7 d (*n* = 58)	Ⅲ: ⩾ 8 d (*n* = 35)
Male / Female	15/21	19/39	16/19
Age (median)	55 (19-76) yr	65 (24-87) yr[b]	62 (30-81) yr[a]
ASA (I/II/III)	18/13/5	23/25/10	14/15/6
Complicated cholecystitis	9	34[b]	15
Preoperative ERCP	2	10	8[a]
Spillage	21	39	18
Drain use	17	32	19
Operative time	55 (35-90) min	62.5 (25-120) min	72.5 (35-120) min[a]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* group I.

Our findings are in accordance with previous studies, suggesting the safety of early LC for AC. The present study, however, does not support the findings of earlier reports, regarding the rising conversion rate, when LC for AC is performed after 72 h[6,12,15]. Our data have shown that the timing of cholecystectomy does not influence the conversion rate, as recently shown by others[8,10]. This is probably attributed to the very low conversion rate in the whole group of our patients, making any differences between the subgroups insignificant. Our total conversion rate of 4.6% for LC in AC is one of the lowest in the literature and only slightly higher than that in our team's experience with elective LC for the same period (~1%). Even one week after the onset of symptoms there was nothing to suggest increased risk with regards to the conversion rate and morbidity; this has never been challenged before. Another issue of concern in laparoscopic treatment of AC is the presumed increased risk of bile duct injury when the procedure is performed beyond the early edematous phase of the first 48-72 h. Our data do not support this traditional belief, as there was no major bile duct injury in any of the patients. It is possible that the majority of patients with AC who are deferred for interval LC because they are outside this "early window of chance" are faced with a "difficult" elective cholecystectomy after few weeks[16]. Waiting for the gallbladder to "cool down" allows maturation of acute inflammation, neovasculaturization, fibrosis, and contraction, making the dissection more difficult, as it has been proposed by others[9]. While inflammation in the early stages may not necessarily involve Calot's triangle structures, chronic inflammation may scar and distort it, making dissection in this critical area more difficult and prone to bile duct injuries.

In conclusion, our data show that LC for AC during the index admission is safe and associated with a low morbidity and a low conversion rate. These findings refer not only to those patients who undergo surgical treatment very

Table 3 Complications and their treatment

Group 1: ⩽ 3 d (*n* = 36)	Group 2: 4-7 d (*n* = 58)	Group 3: ⩾ 8 d (*n* = 35)
(1) Subhepatic collection	(2) Bile leaks	(2) Bile leaks
Laparoscopic drainage	ERCP and CBD stent	ERCP and CBD stent
	Spontaneous closure at 48 h	
(1) Bleeding	(1) Subhepatic collections	(1) Bleeding from drain site
Laparotomy d 1	Percutanous CT guided drainage	Drain removal
	Laparotomy after failed percutaneous	
(1) Wound infection (converted)	(1) Re-admission at postop day 15 with cholangitis	(1) Severe pancreatitis
Wound opening	ERCP & sphincterotomy	ICU admission
	(1) Chest infection	(1) Readmission at postop d 6 with DVT
	Antibiotics, physiothertpy	Heparin

Complications are presented with parentheses to indicate the number of patients suffered the complication; under the complication line the way of management for each case (without numbers) is presented.

early, but also to those treated after the window of the first 3 or 7 d from the onset of symptoms. Further prospective randomized trials focusing on this particular question are required to validate these results. However, it appears reasonable to state that in units with expertise in laparoscopic surgery, every effort should be made to operate on all patients with AC during the index admission as soon as diagnosis is made and co-morbidities are dealt with, regardless of the time delay from the onset of symptoms. This policy is safe, not associated with a higher conversion rate or morbidity and results in an overall shorter hospitalization by avoiding re-admissions.

REFERENCES

1 **Hunter JG**. Acute cholecystitis revisited: get it while it's hot. *Ann Surg* 1998; **227**: 468-469
2 **Lo CM**, Liu CL, Fan ST, Lai EC, Wong J. Prospective randomized study of early versus delayed laparoscopic cholecystectomy for acute cholecystitis. *Ann Surg* 1998; **227**: 461-467
3 **Lai PB**, Kwong KH, Leung KL, Kwok SP, Chan AC, Chung SC, Lau WY. Randomized trial of early versus delayed laparoscopic cholecystectomy for acute cholecystitis. *Br J Surg* 1998; **85**: 764-767
4 **Kolla SB**, Aggarwal S, Kumar A, Kumar R, Chumber S, Parshad R, Seenu V. Early versus delayed laparoscopic cholecystectomy for acute cholecystitis: a prospective randomized trial. *Surg Endosc* 2004; **18**: 1323-1327
5 **Lahtinen J**, Alhava EM, Aukee S. Acute cholecystitis treated by early and delayed surgery. A controlled clinical trial. *Scand J Gastroenterol* 1978; **13**: 673-678
6 **Eldar S**, Eitan A, Bickel A, Sabo E, Cohen A, Abrahamson J, Matter I. The impact of patient delay and physician delay on the outcome of laparoscopic cholecystectomy for acute cholecystitis. *Am J Surg* 1999; **178**: 303-307
7 **Sharp KW**. Acute cholecystitis. *Surg Clin North Am* 1988; **68**: 269-279
8 **Bhattacharya D**, Senapati PS, Hurle R, Ammori BJ. Urgent versus interval laparoscopic cholecystectomy for acute cholecystitis: a comparative study. *J Hepatobiliary Pancreat Surg* 2002; **9**: 538-542
9 **Madan AK**, Aliabadi-Wahle S, Tesi D, Flint LM, Steinberg SM. How early is early laparoscopic treatment of acute cholecystitis? *Am J Surg* 2002; **183**: 232-236
10 **Knight JS**, Mercer SJ, Somers SS, Walters AM, Sadek SA, Toh SK. Timing of urgent laparoscopic cholecystectomy does not influence conversion rate. *Br J Surg* 2004; **91**: 601-604
11 **Chandler CF**, Lane JS, Ferguson P, Thompson JE, Ashley SW. Prospective evaluation of early versus delayed laparoscopic cholecystectomy for treatment of acute cholecystitis. *Am Surg* 2000; **66**: 896-900
12 **Pessaux P**, Tuech JJ, Rouge C, Duplessis R, Cervi C, Arnaud JP. Laparoscopic cholecystectomy in acute cholecystitis. A prospective comparative study in patients with acute vs. chronic cholecystitis. *Surg Endosc* 2000; **14**: 358-361
13 **Serralta AS**, Bueno JL, Planells MR, Rodero DR. Prospective evaluation of emergency versus delayed laparoscopic cholecystectomy for early cholecystitis. *Surg Laparosc Endosc Percutan Tech* 2003; **13**: 71-75
14 **Uchiyama K**, Onishi H, Tani M, Kinoshita H, Ueno M, Yamaue H. Timing of laparoscopic cholecystectomy for acute cholecystitis with cholecystolithiasis. *Hepatogastroenterology* 2004; **51**: 346-348
15 **Garber SM**, Korman J, Cosgrove JM, Cohen JR. Early laparoscopic cholecystectomy for acute cholecystitis. *Surg Endosc* 1997; **11**: 347-350
16 **Lo CM**, Fan ST, Liu CL, Lai EC, Wong J. Early decision for conversion of laparoscopic to open cholecystectomy for treatment of acute cholecystitis. *Am J Surg* 1997; **173**: 513-517

S- Editor Pan BR **L- Editor** Wang XL **E- Editor** Bai SH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5532-5535
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Long-term outcomes of chronic hepatitis C patients with sustained virological response at 6 months after the end of treatment

Disaya Chavalitdhamrong, Tawesak Tanwandee

Disaya Chavalitdhamrong, Tawesak Tanwandee, Division of Gastroenterology, Department of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
Supported by The Gastroenterological Association of Thailand
Correspondence to: Disaya Chavalitdhamrong, MD, Division of Gastroenterology, Department of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand. disaya104@gmail.com
Telephone: +66-2-4197282 Fax: +66-2-4183001
Received: 2006-03-08 Accepted: 2006-04-21

Abstract

AIM: To assess the clinical, biochemical, and virological outcome during long-term follow-up of chronic hepatitis C patients with sustained virological response following effective antiviral therapy.

METHODS: This study was a retrospective cohort study including 171 sustained responders defined as HCV RNA PCR negative at 6 mo after the end of effective antiviral treatment (SVR-6). Clinical signs and symptoms, biochemical hepatic parameters, ultrasonography and HCV RNA PCR were followed.

RESULTS: Mean follow-up period was 35.38 ± 22.2 mo after the end of treatment. Twenty-seven (15.8%) responders had evidence of cirrhosis before treatment. Forty-eight (28.1%), 107 (62.6%) and 6 (3.5%) patients were genotype 1, 3, and 6 respectively, while 10 patients (5.8%) were unclassified. There were no virological and biochemical relapses during the period of follow-up. None of the patients showed evidence of hepatic decompensation. However, there were 3 patients (1.8%) developing hepatocellular carcinoma at 14, 18, 29 mo after treatment discontinuation, two of whom had evidence of cirrhosis prior to therapy.

CONCLUSION: The study shows that during a follow-up interval for about 3 years in 171 chronic hepatitis C patients with sustained viral response after effective antiviral treatment there were no evident signs of either biochemical or clinical relapse of liver disease in all but three patients who developed hepatocellular carcinoma.

Key words: Chronic hepatitis C; Sustained virological response; Long-term outcome

Chavalitdhamrong D, Tanwandee T. Long-term outcomes of chronic hepatitis C patients with sustained virological response at 6 months after the end of treatment. *World J Gastroenterol* 2006; 12(34): 5532-5535

http://www.wjgnet.com/1007-9327/12/5532.asp

INTRODUCTION

Chronic hepatitis C viral (HCV) infection is well recognized as a major cause of cirrhosis and hepatocellular carcinoma. Spontaneous remission of the disease seems to be rare. After alfa-interferon was used as therapy for HCV infection in 1986[1], Interferon associated with Ribavirin became the standard treatment for this disease. Sustained virological response, defined as HCV RNA PCR negative at 6 mo after the end of treatment (SVR-6), is considered to be a useful predictor for long term response[2], since the probability of a late relapse among sustained responders was only 4.7%-8.7%[2-5], and a sustained response is associated with decreased histological activity on liver biopsy[6,12]. Moreover, the disappearance of detectable plasma HCV RNA has fostered the notion that sustained responders may be cured of the disease[4].

From most of the studies, SVR-6 can be obtained in 40%-60% of individuals infected with genotype 1 and in a higher percentage (75%-85%) of subjects with genotypes 2 and 3[5]. However, there are only few studies reporting long-term outcomes in patients who had SVR-6[1-4,6-8], especially in Asian population. In this study, we assessed the long-term clinical, biochemical and virological outcome of Thai HCV patients with sustained virological response.

MATERIALS AND METHODS

Study design

Chronic hepatitis C patients with SVR-6 from Hepatology Unit, Faculty of Medicine, Mahidol University (Siriraj Hospital), Bangkok, Thailand from 1995 to 2005 were included in this retrospective cohort study.

Patients

The criteria required for the study included: evidence of

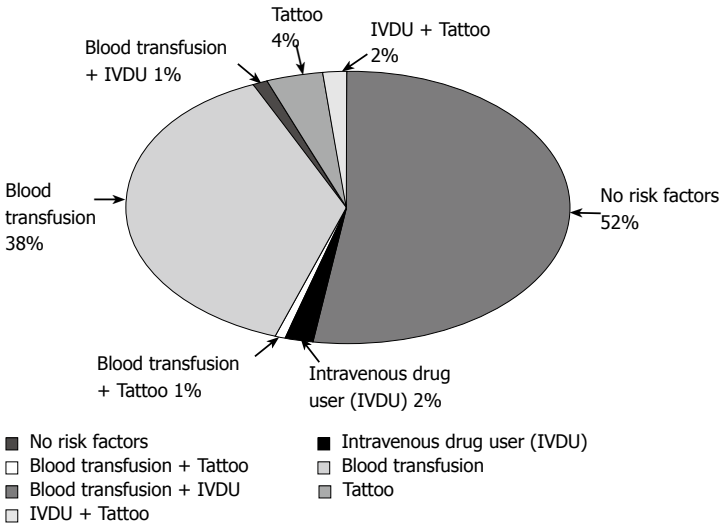


Figure 1 Routes of infection.

Table 1 Characteristics of 171 HCV infection patients with SVR-6 (n = 171)

Characteristics	
Age (yr)	
Mean ± SD	48 ± 10.5
Range	21-75
Sex	
Male	91 (53%)
Female	80 (47%)
Follow up (mo)	
Mean ± SD	35.38 ± 22.2
Range	8-134
Genotype	
1	48 (28.1%)
3	107 (62.6%)
6	6 (3.5%)
Unclassified	10 (5.8%)
Cirrhosis (before therapy)	27 (25.8%)
HCV viral load (copies/mL)	1 000-136 000 000
Median HCV viral load	903 000

chronic HCV infection as defined by HCV RNA PCR positive by Roche Amplicor HCV Assay® (with a detection limit of 100 copies/mL), age of 18 years or older, and sustained virological response after effective antiviral treatment. Patients co-infected by chronic hepatitis B virus and/or human immunodeficiency virus and patients where complete data could not be retrieved were excluded from the analysis.

Data were obtained from 171 chronic hepatitis C patients who responded to interferon therapy. Data were collected from case record forms. Patients treated with conventional interferon, PEG-interferonα-2a and PEG-interferonα-2b were 54.4%, 17% and 28.6% respectively. Twenty-one patients (12.3%) required therapy twice to achieve SVR-6. Duration of therapy was 24 wk for responders with genotype 3 infection, while non-responders and other HCV genotypes patients were treated for 48 wk.

Data recorded

The investigators recorded demographic data, details of treatments (type of interferon used and duration of therapy), virological data (genotypes and viral loads) and biochemical data (bilirubin, transaminases and albumin levels) obtained from certified laboratories. Pre-treatment liver biopsies were done in 105 of the 171 patients using the Histology Activity Index (HAI) score. Mean histological score and fibrosis score were 7.72 ± 4.08 (range 1 to18) and 1.51 ± 1.3 (range 0 to 4) respectively.

Sustained virological response was defined as no detectable HCV RNA PCR at the end of treatment and after six months of follow up.

Patients were considered to have decompensation if they showed any of the following findings: ascites, bleeding varices, jaundice, or hepatic encephalopathy. Patients were classified as having cirrhosis on the basis of ultrasonography or liver biopsy. Hepatocellular carcinoma was diagnosed from ultrasonography or other imaging studies.

Sustained virological responders were considered to have a late virological relapse if HCV RNA PCR became detectable on any occasion after six months of follow up, confirmed by HCV viral load study.

Follow-up data was recorded every six or twelve months for clinical, biochemical and virological outcomes.

Statistical analysis

Statistical analyses were performed using SPSS version 10 for Windows. The descriptive statistics and Paired t-Test were used to analyse. The Kaplan-Meier method was used to determine the rate of hepatocellular carcinoma occurrence.

RESULTS

Study population

Data were obtained from 171 patients treated for chronic HCV infection who had SVR-6. The ages of the patients were 48 ± 10.5 years. Male to female ratio was 1.12:1. Routes of infection are shown in Figure 1. Characteristics of sustained virological responders are shown in Table 1.

Of 171 sustained responders, 27 patients (15.8%) had compensated cirrhosis (as determined by liver biopsy or imaging study) before the start of treatment.

Clinical, biochemical and virological studies were followed up to 35.38 ± 22.2 mo (range 8 to 134 mo) after the end of treatment.

Clinical outcomes

At the end of follow-up, all patients were fully active and alive. No new cases of cirrhosis appeared. Of the 27 patients with cirrhosis, none developed decompensated liver disease. Hepatocellular carcinoma, as assessed by abdominal ultrasonography every 6 mo were found in three patients at 14, 18 and 29 mo of follow-up. The details of these patients are shown in Table 2. Their HCV RNA values were still undetectable at the time of the diagnosis

Table 2 Features of the three hepatocellular carcinoma patients

	Case1	Case 2	Case 3
Age (yr)	50	75	63
Sex	Male	Male	Male
Previous cirrhosis	Yes	Yes	No
Onset after the end of treatment (mo)	14	18	29
Alfa-fetoprotein (IU/mL)	433.7	2.09	21.59
Size at first detection	3 cm	1.5 cm	1 cm, 3 lesions

of hepatocellular carcinoma. Two of them had cirrhosis before treatment. From the statistical analysis of this data, it showed that, after a median follow-up period of 31 mo, the incidence of developing hepatocellular carcinoma in SVR-6 patients was no more than 1.8%.

Biochemical and virological outcomes

There was a significant reduction of aminotransferase levels after SVR-6. The pre-treatment and post-treatment values of AST were 84.3 ± 48.9 and 27.7 ± 12.9, while ALT were 120.4 ± 81.2 and 25.2 ± 15.3 U/L (the normal values of AST: 0-37 and ALT 0-40 U/L).

No late virological relapse was found. Four (2.3%) of the 171 sustained responders had false positive HCV RNA PCR (confirmed by HCV RNA viral load less than 50 IU/mL).

DISCUSSION

We assessed the long term outcome of 171 patients with chronic hepatitis C who achieved sustained virological response by effective antiviral treatment. Our result demonstrated that SVR-6 was associated with a permanent absence of HCV viremia during the long-term follow-up in all cases. However, this finding showed better result than previous reports which had a late virological relapse between 4.7%-8.7%[2,3]. The reasons may due to: (1) the smaller number of patients included compared to previous studied ; (2) fewer HCV genotype 1 included compared; (3) more than half of our patients were infected by genotype 3 (this type is known to have a more favorable prognosis), and (4), the low sensitivity of HCV RNA PCR tests used in the studies before 2000 that may potentially account for few apparent early virological responses.

Disease progression such as development of cirrhosis seems to arrest after the stage of SVR-6, but a risk of developing hepatocellular carcinoma is still remaining. The incidence of hepatocellular carcinoma from our study at a mean follow-up period about 3 years is 1.8% which is slightly higher than the previous results reported from Japan (0.02%-0.5% per year)[3,9,10], while the incidence of hepatocellular carcinoma in western countries seems to be rare after SVR and limited only to patients with cirrhosis[4,11]. Therefore, regular ultrasonography should not be discarded for the management of cirrhotic patients, even in those showing persistently normal aminotransferase, alfa-fetoprotein and undetectable HCV RNA levels after interferon treatment.

In summary, this long-term study shows that in chronic HCV infection, sustained responders to interferon attain remarkable improvement of clinical outcomes. This supports the hypothesis that persons with sustained responses to interferon-therapy show a low risk for further relapse of HCV infection, development of cirrhosis and hepatocellular carcinoma[3,4,6,7,12]. However, the follow-up period of these patients was too short to allow a definite conclusion about the potential effect of the sustained response to interferon therapy for prevention of progressive liver disease .

REFERENCES

1 **Reichard O**, Glaumann H, Frydén A, Norkrans G, Wejstål R, Weiland O. Long-term follow-up of chronic hepatitis C patients with sustained virological response to alpha-interferon. *J Hepatol* 1999; **30**: 783-787

2 **Cammà C**, Giunta M, Pinzello G, Morabito A, Verderio P, Pagliaro L. Chronic hepatitis C and interferon alpha: conventional and cumulative meta-analyses of randomized controlled trials. *Am J Gastroenterol* 1999; **94**: 581-595

3 **Veldt BJ**, Saracco G, Boyer N, Cammà C, Bellobuono A, Hopf U, Castillo I, Weiland O, Nevens F, Hansen BE, Schalm SW. Long term clinical outcome of chronic hepatitis C patients with sustained virological response to interferon monotherapy. *Gut* 2004; **53**: 1504-1508

4 **Bruno S**, Battezzati PM, Bellati G, Manzin A, Maggioni M, Crosignani A, Borzio M, Solforosi L, Morabito A, Ideo G, Podda M. Long-term beneficial effects in sustained responders to interferon-alfa therapy for chronic hepatitis C. *J Hepatol* 2001; **34**: 748-755

5 **Teoh NC**, Farrell GC. Management of chronic hepatitis C virus infection: a new era of disease control. *Intern Med J* 2004; **34**: 324-337

6 **Yoshida H**, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, Yano M, Tanaka M, Fujiyama S, Nishiguchi S, Kuroki T, Imazeki F, Yokosuka O, Kinoyama S, Yamada G, Omata M. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. *Ann Intern Med* 1999; **131**: 174-181

7 **Larghi A**, Tagger A, Crosignani A, Ribero ML, Bruno S, Portera G, Battezzati PM, Maggioni M, Fasola M, Zuin M, Podda M. Clinical significance of hepatic HCV RNA in patients with chronic hepatitis C demonstrating long-term sustained response to interferon-alpha therapy. *J Med Virol* 1998; **55**: 7-11

8 **Marcellin P**, Boyer N, Gervais A, Martinot M, Pouteau M, Castelnau C, Kilani A, Areias J, Auperin A, Benhamou JP, Degott C, Erlinger S. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-alpha therapy. *Ann Intern Med* 1997; **127**: 875-881

9 **Kasahara A**, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, Iijima A, Urushihara A, Kiyosawa K, Okuda M, Hino K, Okita K. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology* 1998; **27**: 1394-1402

10 **Imai Y**, Kawata S, Tamura S, Yabuuchi I, Noda S, Inada M, Maeda Y, Shirai Y, Fukuzaki T, Kaji I, Ishikawa H, Matsuda Y, Nishikawa M, Seki K, Matsuzawa Y. Relation of interferon therapy and hepatocellular carcinoma in patients with chronic hepatitis C. Osaka Hepatocellular Carcinoma Prevention Study Group. *Ann Intern Med* 1998; **129**: 94-99

11 **McHutchison JG**, Esteban-Mur R. Durability of sustained virological response in patients with chronic hepatitis C after

treatment with interferon a-2B alone or in combination with ribavirin. *Hepatology* 2001; **34**: 244A

12 **Shiratori Y**, Imazeki F, Moriyama M, Yano M, Arakawa Y, Yokosuka O, Kuroki T, Nishiguchi S, Sata M, Yamada G, Fujiyama S, Yoshida H, Omata M. Histologic improvement of fibrosis in patients with hepatitis C who have sustained response to interferon therapy. *Ann Intern Med* 2000; **132**: 517-524

S- Editor Wang J **L- Editor** Chiarioni G **E- Editor** Bi L

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5536-5539
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Comparative study of two bowel preparaton regimens for colonoscopy: Senna tablets *vs* sodium phosphate solution

Savit Kositchaiwat, Weerapat Suwanthanmma, Ronnarat Suvikapakornkul, Vaewvadee Tiewthanom, Prisna Rerkpatanakit, Chaowalitr Tinkornrusmee

Kositchaiwat Savit, Suwanthamma Weerapat, Suvikapakornkul Ronnarat, Department of Surgery, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok Province, Thailand
Vaewvadee Tiewthanom, Prisna Rerkpatanakit, Chaowalitr Tinkornrusmee, Department of Nursing Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok Province, Thailand
Co-first author: Weerapat Suwanthanma
Co-correspondence: Ronnarat Suvikapakornkul
Correspondence to: Dr. Savit Kositchaiwat, Department of Surgery, Faculty of Medicine, Ramathibodi Hospital, Praram 6 street, Bangkok Province, Thailand. raskc@mahidol.ac.th
Telephone: +66-2-2011527 Fax: +66-2-2011527
Received: 2006-03-10 Accepted: 2006-03-27

Abstract

AIM: To compare the efficacy and acceptance of senna tablet and sodium phosphate solution for bowel preparation before colonoscopy.

METHODS: One hundred and thirty four patients, who needed elective colonoscopy, were randomly allocated to take 180 mg senna tablet or 95 mL sodium phosphate solution on the day before colonoscopy. The efficacies of both laxatives were compared using the mean difference of colon-cleanliness score of the rectum, sigmoid segments, descending colon, transverse colon and cecum. The scores were rated by two observers who were blinded to the laxatives administered. The higher score means that the colon is cleaner. The efficacy of both laxatives were equivalent if the 95% confidence interval of the mean difference of the score of colon lie within -1 to +1.

RESULTS: On intention-to-treat analysis, the mean cleanliness scores in the four segments of colon except the cecum were higher in the sodium phosphate group than those in senna group (7.9 ± 1.7 *vs* 8.3 ± 1.5, 8.0 ± 1.8 *vs* 8.5 ± 1.4, 7.9 ± 2.0 *vs* 8.5 ± 1.3, 7.9 ± 2.0 *vs* 8.2 ± 1.4 and 7.2 ± 1.7 *vs* 6.9 ± 1.4, respectively). The 95% confidence intervals (95% CI) of mean difference in each segment of colon were not found to lie within 1 point which indicated that their efficacies were not equivalent. The taste of senna was better than sodium phosphate solution. Also, senna had fewer side effects.

CONCLUSION: The efficacy of senna is not equivalent to sodium phosphate solution in bowel preparation for colonoscopy, but senna may be considered an alternative laxative.

Key words: Senna tablet; Sodium phosphate solution; Colonoscopy

Kositchaiwat S, Suwanthanmma W, Suvikapakornkul R, Tiewthanom V, Rerkpatanakit P, Tinkornrusmee C. Comparative study of two bowel preparaton regimens for colonoscopy: Senna tablets *vs* sodium phosphate solution. *World J Gastroenterol* 2006; 12(34): 5536-5539

http://www.wjgnet.com/1007-9327/12/5536.asp

INTRODUCTION

Adequately cleansed colon is essential for colonoscopy. Inadequate bowel preparation might lead to missed diagnosis, increasing the time of colonoscopy by 7.5%-10.3% and increasing cost 12%-22%[1]. Ness *et al*[2] reported that the incidence of inadequate bowel preparation was 21.7% and 5.4% had poor preparation leading to cancellation or abortion of procedure. Currently, the laxatives of choice for bowel preparation are sodium phosphate solution (NaP) and polyethylene glycol solution (PEG). Despite its efficacy[3-5], phosphate solution has poor taste. It may cause electrolyte imbalance, severe nausea and vomiting. The advantage of PEG is its minimal effect on intravascular volume and serum electrolyte balance, but this large-volume laxative is difficult for many patients to tolerate. Although PEG and NaP are equally effective in colonic cleansing[6], NaP is better tolerated. However, NaP may be contraindicated in certain patient populations. The selection of a colonoscopy preparation requires clinical judgment, cost and informed patient preference[7,8].

Senna (*Cassia angustifolia Vahl, Leguminosae*, Indian senna, Tinnevelly senna) is a laxative that stimulates the intestinal motility and affects epithelial transport of water and electrolytes. The main advantages of senna are low cost, safety and ease of ingestion. It had been combined with other laxatives for bowel preparation, and their efficacy ranged from 70% to 85%[9-12]. There are few studies on the efficacy of high-dose senna tablet alone. The aim of

this study was to compare the efficacy and acceptance of senna tablet and sodium phosphate solution for bowel preparation before colonoscopy.

MATERIALS AND METHODS

The study was carried out as a randomized, controlled (equivalent), single-blind trial from June to November 2003. The study population consisted of adult patients who required elective colonoscopy. The exclusion criteria were: (1) known allergy to senna or sodium phosphate solution; (2) presence of severe metabolic, renal and cardiac conditions; (3) bed-ridden or psychotic patient; (4) pregnancy; (5) patient taking laxatives within one week prior to enrollment; and (6) patients who had previous colonic resection surgery. The study was approved by the Ethical Committee Board of the hospital.

The patients were allocated into two groups and they were advised to take full liquid diet two days before colonoscopy. The control group took sodium phosphate solution (Swift® 90 mL, Berlin Pharmaceutical Industry Co. Ltd., Thailand). The experimental group took senna tablet, 180 mg (24 tablets of 7.5 mg /tab, Senokot®, Reckitt Benckiser, Thailand). The patients took the laxatives in divided doses at 14.00 pm and 16.00 pm on the day before colonoscopy. Since the duration of action of NaP is within 6 h, so the laxatives should not interfere the patient's sleeping time.

Data collection

Shortly before colonoscopy, nurses interviewed each patient to assess compliance, acceptance and side effects of laxatives by using visual analog scale. The colonoscopist and his assistant independently rated the quality of bowel cleansing, using visual analog score (VAS) as followed: 0-2 = numerous solid feces, 3-5 = semi-solid feces, 6-7 large volume of liquid feces, 8-10 = small volume of clear liquid or no feces. In an equivalent trial[13], it was important to pre-specified that (1) the mean score should lie above seven to assure that both laxatives were effective, and (2) the 95% confidence intervals of the mean difference lie between -1 and +1 VAS score in all segments of colon. The sample size calculation was based on testing equivalence with power 0.8 and 10% drop out[14]. The variance of VAS score from our pilot study was 2.93.

Before data analysis, the 95% limit of agreement[15] of cleansing score between two colonoscopists will be calculated to confirm the agreement on the assumption that the mean score difference between them should lie within two points. The score used for analysis were the average score from two colonoscopists. The VAS score for acceptance and side effects of the two laxatives were analyzed using Student's *t* test. The outcome variables of accepted and side effects of laxatives were also measured using VAS score. The patients were asked to grade the taste of the laxative as follows: 0-2 = hard to ingest, 3-5 = ingested with very bad feelings, 6-7 = easily ingested, and 8-10 = easily ingested with good feelings. The scores of the side effects (nausea and vomiting, abdominal pain, vertigo and sleeplessness) were rated as follows: 0-2 = no symptom, 3-5 = mild symptom, 6-7 = moderate symptom, and 8-10 = severe symptom.

Table 1 Demographic and baseline colonoscopic data of the patients (*n* = 67)

Characteristics of patients	Senna	NaP
Sex (M/F)	22 : 45	30 : 37
Age (yr, mean ± SD)	54.3 ± 12.7	51.6 ± 12.6
Body weight (kg, mean ± SD)	59.1 ± 10.7	61.8 ± 12.6
Constipation (Yes/No)	9:58	14:53
Laxative users (Yes/No)	6:61	12:55
Previous Obs-gyn surgery	6:61	7:60
Diabetes (Yes/No)	7:60	6:61
Colonoscopic diagnosis		
Normal study	44 (65.7%)	40 (62.5%)
Polyp	8 (12.1%)	4 (6.2%)
Diverticulosis	4 (6.0%)	8 (12.5%)
Carcinoma	4 (6.0%)	6 (9.3%)
Inflammatory bowel disease	6 (9.0%)	3 (4.6%)
Other	1 (1.5%)	3 (4.6%)
Time of colonoscopy (min, mean ± SD)	19.3 ± 14.2	18.2 ± 10.1
Incomplete colonoscopy	4 (6.0%)	5 (7.8%)
Therapeutic:Diagnostic colonoscopy	16:51	14:50

Table 2 The cleansing score, acceptance score and side effects of laxatives (*n* = 67)

Segment	Senna (mean ± SD)	NaP (mean ± SD)	95% CI of differences
Rectum	7.9 ± 1.7	8.3 ± 1.5	- 1.0 to 0.1
Sigmoid colon	8.0 ± 1.8	8.5 ± 1.4	- 1.0 to 0.1
Descending colon	7.9 ± 2.0	8.5 ± 1.3	- 1.2 to 0.0
Transverse colon	7.9 ± 2.0	8.2 ± 1.4	- 0.9 to 0.3
Ascending colon and cecum	7.2 ± 1.7	6.9 ± 1.4	- 0.2 to 0.8
Acceptance score			
Taste	8.6 ± 1.9	5.1 ± 2.8	$P < 0.001$
Side effects			
Nausea & vomiting	0.9 ± 0.2	3.0 ± 3.5	$P < 0.001$
Abdominal pain	1.3 ± 2.3	1.4 ± 2.4	$P = 0.8$
Vertigo	0.7 ± 1.6	1.3 ± 2.3	$P = 0.08$
Sleeplessness	1.2 ± 2.7	1.4 ± 2.5	$P = 0.65$
Adverse event	2:67	2:64	

RESULTS

Patient's flow in this study is shown in Figure 1. One hundred and seventy patients were enrolled, but thirty patients did not meet eligibility criteria. One hundred and thirty-six patients were randomly allocated to take senna tablet or NaP solution. Two patients did not take laxatives due to error in packaging. Among the 134 patients who took a laxative, 3 patients did not attend colonoscopy and 10 patients did not have complete colonoscopy for various reasons.

Both groups of patients were comparable with regard to demographic data, diagnosis and other colonoscopic data (Table 1). However, the efficacy of senna tablet was not equivalent to NaP solution (Table 2). The mean

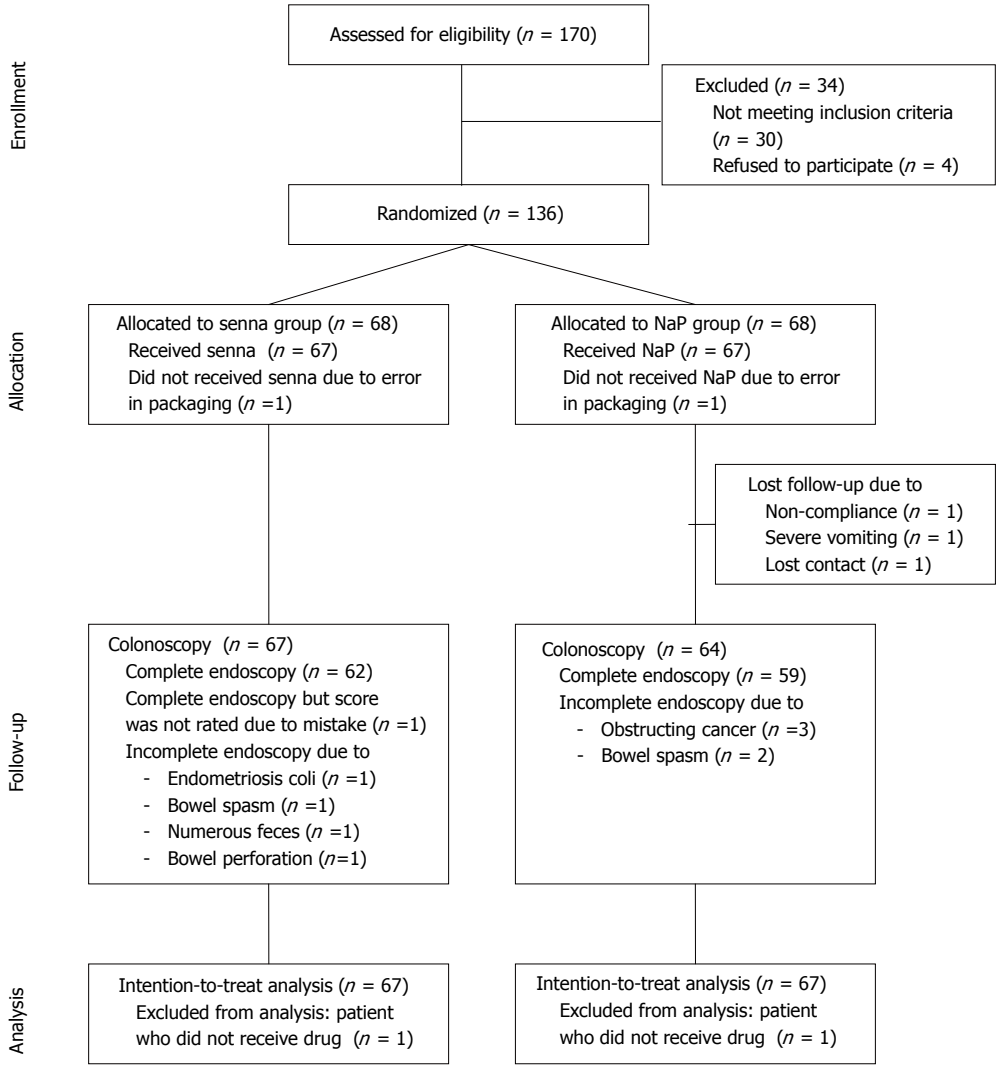


Figure 1 Flow diagram of patients progress through the phases of a randomized trial.

cleansing scores of NaP solution were higher than senna tablet in four segments of the colon except in the ascending colon and the cecum. The 95% CI of the mean difference exceeded 1 point in three segments of the colon. In the other two segments of the colon, they lied nearly over 1 point. By intention-to-treat analysis, we included all patients who had taken the laxatives whether they had complete or incomplete colonoscopy. For the missing data in both groups, we assigned the lowest score in each group (worst-case approach). For example, the lowest cleansing score in senna group was two, while that in NaP group was four.

The cleansing score, acceptance score and side effects of the two laxatives are shown in Table 2. The patients accepted senna tablets more than NaP solution and those patients who took senna tablets had less nausea and vomiting. There were four adverse events in this study. In the senna group, 1 patient had post-polypectomy bleeding which ceased spontaneously, and 1 patient had sigmoid perforation during colonoscopy due to fixation of the sigmoid colon; this patient had received long-term steroid treatment of myasthenia gravis and also had previous left hip surgery. In the NaP group, 2 patients had bronchospasm after colonoscopy and both recovered after 24 h.

DISCUSSION

NaP solution and PEG had widely been used for bowel preparation because of their similar efficacy, Hwang *et al*[16] claimed that NaP group had higher completion rate than PEG group (84.2% *vs* 27.5%, $P < 0.001$) and NaP appeared to be more cost-effective[16]. In contrast, senna was not popular for bowel preparation. Fear of adverse effects might responsible for its underuse. Serious adverse effects of senna, such as asthma, hepatitis, hypertrophic osteoarthropathy, cachexia, hypo-gammaglobulinemia, finger clubbing and tetany, had been reported[17-21]. However, these adverse effects were uncommon and resulted from long-term and large amount used. There are no epidemiologic data to support neoplastic potential of senna compound[22]. The inconsistent efficacy of senna might be another reason for its underuse. Two studies by Chilton *et al*[11] and Valverde *et al*[23] showed that senna (X-prep) solution alone or senna in combination with other laxatives were better than PEG or NaP solution. On contrary, two other studies by Dahshan *et al*[24] and Arezzo *et al*[12] showed that

those standard laxatives were better than senna. Moreover, Hangartner *et al*[9] and Borkje *et al*[10] concluded that senna has no clinical difference compared with those laxatives. Radaelli *et al*[25,26] had claimed that high-dose senna had 97.3% efficacy in bowel cleansing, and that 288 mg of senna was better than 4 L of PEG-ES (90.6% *vs* 79.7% efficacy, $P = 0.003$). In contrast, our study showed that 180 mg of senna tablets did not have equivalent efficacy as NaP solution. The inconsistencies of these results were hardly explained. Bowel cleansing may be affected by other factors, such as gender, age, obesity, race, constipation, previous abdominal surgery and associated complicated diverticular disease[27]. In addition, the mean score of senna group was also above seven points and we imply that senna has some effect in bowel cleansing and it may be alternative laxative for bowel preparation.

In addition, we noticed that the mean cleansing score of cecum in the senna group was higher than that in the NaP group. This phenomenon might be related to timing of laxative intake. Church *et al*[28] suggested that the patients who took laxatives 5 h before colonoscopy had better result than patients who took laxative 1 d before colonoscopy. The VAS scores of taste, nausea and vomiting in the senna group were significantly better compared with the NaP group (Table 2). However, in term of pain symptom, senna was not found to be better than NaP. These findings confirmed our rational background knowledge that senna had more palatability and less nausea and vomiting than NaP solution. The adverse events occurred in 4 patients were not related to laxatives but were related to colonoscopy or anesthetic procedure.

In conclusion, senna does not have the same efficacy as oral NaP solution. However, senna has better compliance and fewer side effects than NaP. Senna may be prescribed as an alternative laxative for bowel preparation in patients who have contraindications to NaP solution.

REFERENCES

1 **Rex DK**, Imperiale TF, Latinovich DR, Bratcher LL. Impact of bowel preparation on efficiency and cost of colonoscopy. *Am J Gastroenterol* 2002; **97**: 1696-1700

2 **Ness RM**, Manam R, Hoen H, Chalasani N. Predictors of inadequate bowel preparation for colonoscopy. *Am J Gastroenterol* 2001; **96**: 1797-1802

3 **Vanner SJ**, MacDonald PH, Paterson WG, Prentice RS, Da Costa LR, Beck IT. A randomized prospective trial comparing oral sodium phosphate with standard polyethylene glycol-based lavage solution (Golytely) in the preparation of patients for colonoscopy. *Am J Gastroenterol* 1990; **85**: 422-427

4 **Kolts BE**, Lyles WE, Achem SR, Burton L, Geller AJ, MacMath T. A comparison of the effectiveness and patient tolerance of oral sodium phosphate, castor oil, and standard electrolyte lavage for colonoscopy or sigmoidoscopy preparation. *Am J Gastroenterol* 1993; **88**: 1218-1223

5 **Cohen SM**, Wexner SD, Binderow SR, Nogueras JJ, Daniel N, Ehrenpreis ED, Jensen J, Bonner GF, Ruderman WB. Prospective, randomized, endoscopic-blinded trial comparing precolonoscopy bowel cleansing methods. *Dis Colon Rectum* 1994; **37**: 689-696

6 **Hsu CW**, Imperiale TF. Meta-analysis and cost comparison of polyethylene glycol lavage versus sodium phosphate for colonoscopy preparation. *Gastrointest Endosc* 1998; **48**: 276-282

7 **Nelson DB**, Barkun AN, Block KP, Burdick JS, Ginsberg GG, Greenwald DA, Kelsey PB, Nakao NL, Slivka A, Smith P, Vakil N. Technology Status Evaluation report. Colonoscopy preparations. May 2001. *Gastrointest Endosc* 2001; **54**: 829-832

8 **Toledo TK**, DiPalma JA. Review article: colon cleansing preparation for gastrointestinal procedures. *Aliment Pharmacol Ther* 2001; **15**: 605-611

9 **Hangartner PJ**, Münch R, Meier J, Ammann R, Bühler H. Comparison of three colon cleansing methods: evaluation of a randomized clinical trial with 300 ambulatory patients. *Endoscopy* 1989; **21**: 272-275

10 **Børkje B**, Pedersen R, Lund GM, Enehaug JS, Berstad A. Effectiveness and acceptability of three bowel cleansing regimens. *Scand J Gastroenterol* 1991; **26**: 162-166

11 **Chilton AP**, O'Sullivan M, Cox MA, Loft DE, Nwokolo CU. A blinded, randomized comparison of a novel, low-dose, triple regimen with fleet phospho-soda: a study of colon cleanliness, speed and success of colonoscopy. *Endoscopy* 2000; **32**: 37-41

12 **Arezzo A**. Prospective randomized trial comparing bowel cleaning preparations for colonoscopy. *Surg Laparosc Endosc Percutan Tech* 2000; **10**: 215-217

13 **Röhmel J**. Therapeutic equivalence investigations: statistical considerations. *Stat Med* 1998; **17**: 1703-1714

14 **Jones B**, Jarvis P, Lewis JA, Ebbutt AF. Trials to assess equivalence: the importance of rigorous methods. *BMJ* 1996; **313**: 36-39

15 **Bland JM**, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* 1986; **1**: 307-310

16 **Hwang KL**, Chen WT, Hsiao KH, Chen HC, Huang TM, Chiu CM, Hsu GH. Prospective randomized comparison of oral sodium phosphate and polyethylene glycol lavage for colonoscopy preparation. *World J Gastroenterol* 2005; **11**: 7486-7493

17 **Marks GB**, Salome CM, Woolcock AJ. Asthma and allergy associated with occupational exposure to ispaghula and senna products in a pharmaceutical work force. *Am Rev Respir Dis* 1991; **144**: 1065-1069

18 **Beuers U**, Spengler U, Pape GR. Hepatitis after chronic abuse of senna. *Lancet* 1991; **337**: 372-373

19 **Armstrong RD**, Crisp AJ, Grahame R, Woolf DL. Hypertrophic osteoarthropathy and purgative abuse. *Br Med J* (Clin Res Ed) 1981; **282**: 1836

20 **Levine D**, Goode AW, Wingate DL. Purgative abuse associated with reversible cachexia, hypogammaglobulinaemia, and finger clubbing. *Lancet* 1981; **1**: 919-920

21 **Prior J**, White I. Tetany and clubbing in patient who ingested large quantities of senna. *Lancet* 1978; **2**: 947

22 **Xing JH**, Soffer EE. Adverse effects of laxatives. *Dis Colon Rectum* 2001; **44**: 1201-1209

23 **Valverde A**, Hay JM, Fingerhut A, Boudet MJ, Petroni R, Pouliquen X, Msika S, Flamant Y. Senna vs polyethylene glycol for mechanical preparation the evening before elective colonic or rectal resection: a multicenter controlled trial. French Association for Surgical Research. *Arch Surg* 1999; **134**: 514-519

24 **Dahshan A**, Lin CH, Peters J, Thomas R, Tolia V. A randomized, prospective study to evaluate the efficacy and acceptance of three bowel preparations for colonoscopy in children. *Am J Gastroenterol* 1999; **94**: 3497-3501

25 **Radaelli F**, Minoli G. Colonoscopy preparation: is there still room for senna? *Gastrointest Endosc* 2002; **56**: 463

26 **Radaelli F**, Meucci G, Imperiali G, Spinzi G, Strocchi E, Terruzzi V, Minoli G. High-dose senna compared with conventional PEG-ES lavage as bowel preparation for elective colonoscopy: a prospective, randomized, investigator-blinded trial. *Am J Gastroenterol* 2005; **100**: 2674-2680

27 **Cirocco WC**, Rusin LC. Factors that predict incomplete colonoscopy. *Dis Colon Rectum* 1995; **38**: 964-968

28 **Church JM**. Effectiveness of polyethylene glycol antegrade gut lavage bowel preparation for colonoscopy--timing is the key! *Dis Colon Rectum* 1998; **41**: 1223-1225

S- Editor Pan BR **L- Editor** Kumar M **E- Editor** Bai SH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5540-5543
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Acute effect of smoking on gallbladder emptying and refilling in chronic smokers and nonsmokers: A sonographic study

Bumin Degirmenci, Ramazan Albayrak, Alpay Haktanir, Murat Acar, Aylin Yucel

Bumin Degirmenci, Ramazan Albayrak, Alpay Haktanir, Murat Acar, Aylin Yucel, Department of Radiology, Faculty of Medicine, Kocatepe University, Afyon, Turkey
Correspondence to: Bumin Degirmenci, Department of Radiology, Faculty of Medicine, Kocatepe University, Afyon, Turkey. bd2002tr@yahoo.com
Telephone: +272-2136707 Fax: +272-2144996
Received: 2005-11-16 Accepted: 2006-01-14

Abstract

AIM: To ultrasonographicaly evaluate the acute effects of smoking on gallbladder contraction and refilling in chronic smokers and nonsmokers.

METHODS: Fifteen chronic smokers (21-30 years old) and fifteen nonsmokers (21-35 years old) participated in this study. Chronic smokers were selected among the volunteers who had been smoking for at least 5 years and 10 cigarettes per day (mean 17.5/d). Examinations were performed in two separate days. In the first day, basal gallbladder (GB) volumes of volunteers were measured after 8-h fasting. After the examinations, participants had a meal containing at least 30-40 gram fat. Gallbladder volume was assessed at 5, 15, 30, 60, 120 and 180 min after the meal. In the second day, participants smoked 2 cigarettes after 8-h fasting. Then, they had the same meal, and gallbladder measurements were repeated at the same time points. Same procedures were applied to both groups.

RESULTS: The mean starving GB volumes were 23.3 ± 3.3 mL in the first day, 21.9 ± 3.0 mL in the second day in nonsmoker group and 18.3 ± 3.0 mL in the first day, 19.5 ± 2.8 mL in second day in smoker group. There was no significant difference between starving GB volumes. We did not find any significant difference between the GB volumes measured at 5, 15, 30, 60, 120 and 180 min in the first and second days in nonsmoker group. In smokers, post cigarette GB volume was found significantly higher at 5, 15 and 30 min which corresponded to GB contraction phase ($P < 0.05$). Control GB volume measurements were not significantly different between the two groups. Post-smoking GB volumes were also not significantly different between the two groups.

CONCLUSION: Smoking prolongs the maximal GB emptying time both in smokers and in nonsmokers though it is not significant. It delays GB contraction in chronic smokers and causes a significant decrease in GB emptying volume. Smoking causes no significant delay in GB refilling in both smokers and nonsmokers. These effects of smoking observed in acute phase result in bile stasis in GB. Bile stasis is the underlying cause of most GB disorders in chronic process.

Key words: Gallbladder; Emptying; Smoking; Ultrasonography

Degirmenci B, Albayrak R, Haktanir A, Acar M, Yucel A. Acute effect of smoking on gallbladder emptying and refilling in chronic smokers and nonsmokers: A sonographic study. *World J Gastroenterol* 2006; 12(34): 5540-5543

http://www.wjgnet.com/1007-9327/12/5540.asp

INTRODUCTION

Smoking is still widespread in many societies despite the recognized relationship of it with many diseases. It is one of the risk factors for lung, stomach, larynx, esophagus, and some other cancers. Its association with lung cancer has been well described. Besides, smoking carries an important risk of developing cardiovascular diseases[1]. Chronic pulmonary diseases, gastric ulcers, and many other diseases are increased among smokers. Some reports showed that smoking increases the risk of gallbladder cancer[2]. Many prospective studies have found an association between smoking and clinical gallbladder disease[3-6]. On the other hand, no relation has been found between smoking and gallstone formation in some other reports[7,8]. A population-based study reported that sonographically detected gallbladder diseases are associated with smoking and are moderately increased in smokers[9]. Some epidemiologic researches focused on the relation of smoking and gallbladder diseases, but they have not found any positive relation[10,11]. There are a limited number of studies in the literature evaluating the effect of smoking on gallbladder motility by ultrasonography. Janderko *et al*[12] evaluated gallbladder contraction and refilling in chronic smokers by ultrasonography and found that refilling is delayed. In our study, we evaluated the gallbladder volume changes in contraction and refilling periods in both smokers and nonsmokers by

ultrasonography. We compared the obtained data of both groups. To our knowledge, there is no other study in the literature evaluating the effects of smoking on gallbladder in the acute period in smokers and nonsmokers by ultrasonography.

MATERIALS AND METHODS

Subjects

Fifteen smoker (10 women and 5 men) and 15 nonsmoker volunteers (9 men and 6 women) were included in the study. The mean age of smokers was 24.2 years (21-30 years), and the mean age of nonsmokers was 28.1 years (21-34 years). Volunteers having smoked for at least 5 years were included in group of smokers. The mean smoking rate in this group was 17.5 cigarettes per day (10-25). None of the volunteers had gastrointestinal disorder, gallbladder disease, any disease like diabetes mellitus that could affect gallbladder, or any previous surgery of gastrointestinal tract. Prior to 48-h and during the study, no medicine or smoking was allowed. A preliminary gallbladder sonography was performed and only the volunteers without any gallbladder abnormality were included in the study. All participants gave their informed consent.

Study design

Gallbladder volumes of all volunteers were measured twice in two separate days. At least 8 h after the last meal, participants were taken into the dimly lighted sonography room at a temperature of 22-24℃. A 3.5 6 MHz convex broadband (tissue harmonic) abdominal transducer (Toshiba, Nemio, Tokyo-Japan) was established for the measurements. All measurements were performed in supine, left lateral, and lateral decubit positions in which gallbladder cross-sectional and longitudinal diameters were best visualized. The best imaging position of gallbladder was recorded for each participant and used in following measurements. The most appropriate transducer position was noted for each volunteer and marked on the skin. Cross-sectional diameters were calculated from the widest diameters measured during sonography. The longest axis of the gallbladder was established as the longitudinal diameter. The distance between internal margins of each opposite wall was used for the measurement of diameters. The subjects with significant folding of gallbladder or the subjects whose longitudinal diameter could not be viewed in a single frame were excluded from the study.

Longitudinal and cross-sectional diameter measurements were repeated three times and the mean of these three measurements was established. Gallbladder volume was calculated with the formula $V = (\pi/6) \times L \times W \times H$, where V = GB volume, L = GB length, W = GB width, H = GB height, as previously described[13].

Study methods

Gallbladder volumes of all volunteers were measured twice in two separate days. In the first day (control examination) after 8-h (at least) overnight fasting, basal GB volumes were measured. Then, 30-40 g fat-containing meal was given to the volunteers and participants were told to eat the meal up in 2 min. After that, GB volumes were measured at 5, 15, 30, 60, 120 and 180 min. Contraction phase as the measurements till 60 min, and refilling phase was presumed as the measurements at 120 and 180 min. To avoid the start of cephalic phase of digestion, volunteers and meals were kept in separate rooms until the examinations. In the second day (study examination), after an overnight fasting, two cigarettes were given to the participants who were encouraged to consume the cigarettes in 5-10 min with deep inhalations. Approximately 3 min after the smoking, a meal containing 30-40 g fat, was given to the volunteers. Then, GB volumes were measured at 5, 15, 30, 60, 120 and 180 min.The same procedures were repeated in smoker and nonsmoker groups.

GB volume changes versus time were presented graphically for both two groups using the pre- and post-smoking values (Figure 1A and B). Acquired data are shown on Tables 1 and 2 by comparing them between the two groups and within each group.

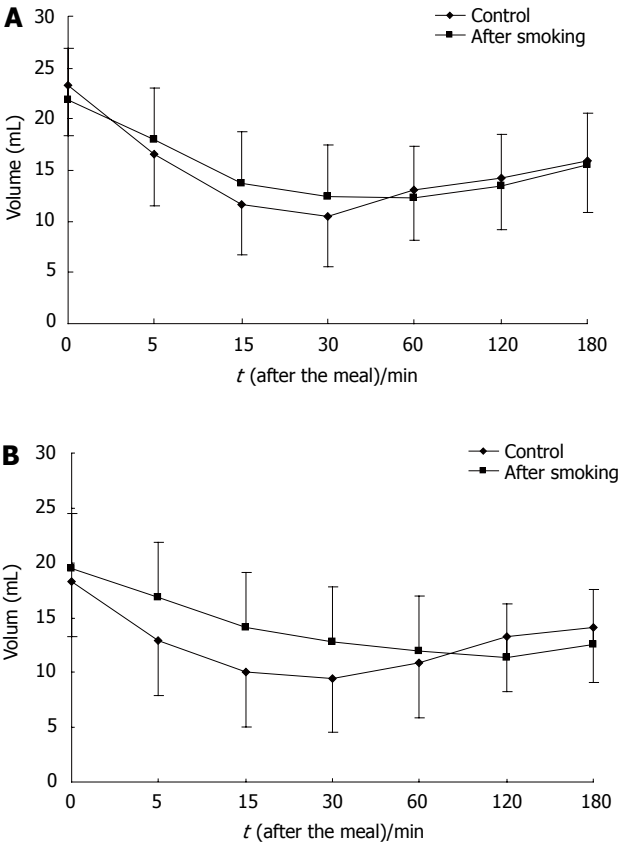


Figure 1 Effect of smoking on gallbladder emptying and refilling in nonsmokers (**A**) and smokers (**B**).

Statistical analysis

Values are presented as mean ± SD. The paired and two-tailed Student's *t* tests were used. $P < 0.05$ was considered statistical significant.

RESULTS

There was no difference in body mass index and age between smokers and nonsmokers. The mean fasting GB

Table 1 Postprandial gallbladder volumes after smoking in smokers and non-smokers (mean ± SD, mL)

	Non smoker			Smoker		
t/min	Control	After smoking	*P*	Control	After smoking	*P*
0	23.3 ± 3.3	21.9 ± 3.0	0.175	18.3 ± 3.0	19.5 ± 2.8	0.371
5	16.5 ± 2.9	18.0 ± 2.8	0.174	12.9 ± 2.1	16.9 ± 2.5	0.022
15	11.7 ± 2.5	13.7 ± 2.6	0.080	10.0 ± 1.5	14.1 ± 1.6	0.011
30	10.5 ± 1.9	12.4 ± 2.1	0.432	9.5 ± 1.6	12.8 ± 1.7	0.042
60	13.1 ± 2.4	12.3 ± 2.0	0.756	10.9 ± 1.5	12.0 ± 1.6	0.359
120	14.2 ± 2.5	13.5 ± 1.8	0.859	13.3 ± 2.9	11.3 ± 1.5	0.398
180	15.9 ± 2.5	15.5 ± 1.9	0.508	14.1 ± 2.9	12.6 ± 1.7	0.449

Table 2 Postprandial gallbladder volumes after smoking in smokers and non-smokers (mean ± SD, mL)

	Control			After smoking		
t/min	Non smoker	Smoker	*P*	Non smoker	Smoker	*P*
0	23.3 ± 3.3	18.3 ± 3.0	0.360	21.9 ± 3.0	19.5 ± 2.8	0.604
5	16.5 ± 2.9	12.9 ± 2.1	0.392	18.0 ± 2.8	16.9 ± 2.5	0.783
15	11.7 ± 2.5	10.0 ± 1.5	0.623	13.7 ± 2.6	14.1 ± 1.6	0.883
30	10.5 ± 1.9	9.5 ± 1.6	0.732	12.4 ± 2.1	12.8 ± 1.7	0.883
60	13.1 ± 2.4	10.9 ± 1.5	0.513	12.3 ± 2.0	12.0 ± 1.6	0.909
120	14.2 ± 2.5	13.3 ± 2.9	0.839	13.5 ± 1.8	11.3 ± 1.5	0.318
180	15.9 ± 2.5	14.1 ± 2.9	0.701	15.5 ± 1.9	12.6 ± 1.7	0.295

volume in nonsmoker group was 23.3 ± 3.3 mL in the first day and 21.9 ± 3.0 mL in the second day, and was 18.3 ± 3.0 mL in the first day and 19.5 ± 2.8 mL in the second day in smoker group. Although the fasting GB volumes did not differ significantly, these values were lower in smoker group. In the first day, minimum GB volumes (in other words, maximal GB emptying) were measured at 30 min in both groups.

However, the post-smoking measurements in the second day were performed at 60 postprandial minute in nonsmoker group, and at 120 min in smoker group (Figures 1A and 1B). Smoking delayed the maximal GB emptying in both smokers and nonsmokers. Besides, in both groups, GB refilling was faster in the first day and slowed down in the second day (Figure 1A and B). In nonsmoker group, GB volumes measured at 5, 15, 30, 60, 120 and 180 min in the first and second days were not significantly different (Table 1). GB volume was significantly higher in smoker group at GB contraction phase (5, 15 and 30 min) after the two cigarettes were smoked ($P < 0.05$, Table 1). There was no significant difference between two groups both in basal GB volume measurements and in post-smoking measurements between the two groups (Table 2).

DISCUSSION

In this study, we showed the disrupted GB contractility in smokers and delayed maximal GB emptying both in smokers and nonsmokers just after smoking. Although our study group was small in size, basal GB volume had a tendency to be lower in smokers than in nonsmokers. However, this difference was not statistically significant. Many epidemiologic studies have reported a mild or moderate association between smoking and gallstone or postcholecystectomic state[4,6,10,11]. On the other hand, no association has been detected between smoking and gall stone formation[7,8,14]. It is not clear which biologic mechanism is mediated in the predisposition of smoking to gallstone formation. Some authors suggested that smoking lowers the plasma high-density lipoprotein cholesterol level which increases risk of gall stone formation by decreasing hepatic excretion of the bile acids[15]. Estrogen is blamed for gall stone formation because of high incidence of gallstone in women. In respect to this, some studies have shown high plasma estrogen level in smokers[16], while some others have not found any significant difference[17]. Cholecystokinin, a proximal gout hormone, is a well known mediator of GB contraction[18]. It is the main determining factor for the postprandial GB discharge. Cholecystokinin starts GB contraction by affecting the pre-ganglionic cholinergic nerves. Cholecystokinin is released from mucosa cells by the arrival of stomach content that is rich in fat and protein to the small intestine[19]. Smoking exerts an inhibitory effect on intestinal and gastric motility[20-22]. As a result of decreased gut motility and delayed gastric emptying, cholecystokinin release is also decreased or delayed. Therefore, decreased GB contractility can be expected. However, in our study, smoking caused a nonsignificant and minimal decrease of GB contractility in nonsmoker volunteers. In addition, GB volume was slightly increased in post-smoking contraction phase in this group (Figure 1A). Previous studies have also reported similar results[23]. On the other hand, we showed a significant difference of GB volume in post-smoking early contraction phase (postprandial 5, 15 and 30 min) of smokers (Table 2). It was also significantly higher in post-smoking 5, 15 and 30 min (Figure 1B). To the best of our knowledge, no data are available on whether this delay of GB contraction in smokers results from a disruption in the smooth muscle contraction mechanism of GB wall or from the delay of the blood cholecystokinin increase due to delayed gastric emptying. One of the limitations of this study is that the blood levels of cholecystokinin were not measured. According to Jonderko *et al*[12], smoking delays GB refilling at acute phase in chronic smokers. Our results also showed that GB refilling was delayed after smoking both in smokers and in nonsmokers although not significant. Smoking suppresses pancreatic polypeptide release which plays an important role in GB refilling[24,25].

In humans, disruption of GB emptying is associated with gallstone formation[26]. Bile stasis and disrupted GB motility are important factors for gallstone formation[27,28]. Delayed gallbladder emptying and reduced muscle contractility occur in chronic calculus, or in gallstone, cholecystitis[29,30]. It was reported that acalculous cholecystitis is formed after inhalation of intense cigarette smoke in dogs[31]. Impairment of gallbladder contractility may contribute to the clinicopathology of acalculous cholecystitis[32].

In our study, smoking caused a nonsignificant prolongation of the maximal GB emptying time both in smokers and in nonsmokers at acute phase, delayed GB contraction in smokers, and decreased the GB emptying volume. Besides, smoking did not delay GB refilling

significantly in both groups. Because of all these acute effects, smoking comprises a risk for GB diseases. In the chronic process, smoking may have these effects by affecting GB smooth muscle contraction or by decreasing cholecystokinin release *via* the inhibition of intestinal motility, or by both. *In vitro* studies are needed to evaluate the effects of smoking on gallbladder smooth muscles and laboratory studies are required to show its effects on cholecystokinin release.

REFERENCES

1 **Jonas MA**, Oates JA, Ockene JK, Hennekens CH. Statement on smoking and cardiovascular disease for health care professionals. American Heart Association. *Circulation* 1992; **86**: 1664-1669
2 **Khan ZR**, Neugut AI, Ahsan H, Chabot JA. Risk factors for biliary tract cancers. *Am J Gastroenterol* 1999; **94**: 149-152
3 **Diehl AK**, Haffner SM, Hazuda HP, Stern MP. Coronary risk factors and clinical gallbladder disease: an approach to the prevention of gallstones? *Am J Public Health* 1987; **77**: 841-845
4 **Kono S**, Shinchi K, Ikeda N, Yanai F, Imanishi K. Prevalence of gallstone disease in relation to smoking, alcohol use, obesity, and glucose tolerance: a study of self-defense officials in Japan. *Am J Epidemiol* 1992; **136**: 787-794
5 **Murray FE**, Logan RF, Hannaford PC, Kay CR. Cigarette smoking and parity as risk factors for the development of symptomatic gall bladder disease in women: results of the Royal College of General Practitioners' oral contraception study. *Gut* 1994; **35**: 107-111
6 **Kato I**, Nomura A, Stemmermann GN, Chyou PH. Prospective study of clinical gallbladder disease and its association with obesity, physical activity, and other factors. *Dig Dis Sci* 1992; **37**: 784-790
7 **Chen CY**, Lu CL, Lee PC, Wang SS, Chang FY, Lee SD. The risk factors for gallstone disease among senior citizens: an Oriental study. *Hepatogastroenterology* 1999; **46**: 1607-1612
8 **Attili AF**, Capocaccia R, Carulli N, Festi D, Roda E, Barbara L, Capocaccia L, Menotti A, Okolicsanyi L, Ricci G, Lalloni L, Mariotti S, Sama C, Scafato E. Factors associated with gallstone disease in the MICOL experience. Multicenter Italian Study on Epidemiology of Cholelithiasis. *Hepatology* 1997; **26**: 809-818
9 **Misciagna G**, Leoci C, Guerra V, Chiloiro M, Elba S, Petruzzi J, Mossa A, Noviello MR, Coviello A, Minutolo MC, Mangini V, Messa C, Cavallini A, De Michele G, Giorgio I. Epidemiology of cholelithiasis in southern Italy. Part II: Risk factors. *Eur J Gastroenterol Hepatol* 1996; **8**: 585-593
10 **Okamoto M**, Yamagata Z, Takeda Y, Yoda Y, Kobayashi K, Fujino MA. The relationship between gallbladder disease and smoking and drinking habits in middle-aged Japanese. *J Gastroenterol* 2002; **37**: 455-462
11 **Kono S**, Eguchi H, Honjo S, Todoroki I, Oda T, Shinchi K, Ogawa S, Nakagawa K. Cigarette smoking, alcohol use, and gallstone risk in Japanese men. *Digestion* 2002; **65**: 177-183
12 **Jonderko K**, Nowak A, Kasicka-Jonderko A, Błaszczyńska M. Effect of cigarette smoking on gallbladder emptying and filling in man. *Am J Gastroenterol* 1994; **89**: 67-71
13 **Dodds WJ**, Groh WJ, Darweesh RM, Lawson TL, Kishk SM, Kern MK. Sonographic measurement of gallbladder volume. *AJR Am J Roentgenol* 1985; **145**: 1009-1011
14 **Layde PM**, Vessey MP, Yeates D. Risk factors for gall-bladder disease: a cohort study of young women attending family planning clinics. *J Epidemiol Community Health* 1982; **36**: 274-278
15 **Criqui MH**, Wallace RB, Heiss G, Mishkel M, Schonfeld G, Jones GT. Cigarette smoking and plasma high-density lipoprotein cholesterol. The Lipid Research Clinics Program Prevalence Study. *Circulation* 1980; **62**: IV70- IV76
16 **Khaw KT**, Tazuke S, Barrett-Connor E. Cigarette smoking and levels of adrenal androgens in postmenopausal women. *N Engl J Med* 1988; **318**: 1705-1709
17 **Dai WS**, Gutai JP, Kuller LH, Laporte RE, Falvo-Gerard L, Caggiula A. Relation between plasma high-density lipoprotein cholesterol and sex hormone concentrations in men. *Am J Cardiol* 1984; **53**: 1259-1263
18 **Niebergall-Roth E**, Teyssen S, Singer MV. Neurohormonal control of gallbladder motility. *Scand J Gastroenterol* 1997; **32**: 737-750
19 **Shaffer EA**. Review article: control of gall-bladder motor function. *Aliment Pharmacol Ther* 2000; **14** Suppl 2: 2-8
20 **Watanabe Y**, Fujiwara Y, Shiba M, Watanabe T, Tominaga K, Oshitani N, Matsumoto T, Nishikawa H, Higuchi K, Arakawa T. Cigarette smoking and alcohol consumption associated with gastro-oesophageal reflux disease in Japanese men. *Scand J Gastroenterol* 2003; **38**: 807-811
21 **Nowak A**, Jonderko K, Kaczor R, Nowak S, Adamczak D, Rudzki K. Inhibitory effect of cigarette smoking on gastric emptying of a solid meal in patients with type I gastric ulcer. *Z Gastroenterol* 1991; **29**: 45-48
22 **Miller G**, Palmer KR, Smith B, Ferrington C, Merrick MV. Smoking delays gastric emptying of solids. *Gut* 1989; **30**: 50-53
23 **Jonderko K**, Sliwinski Z, Lelek A, Kaczor R. Effect of cigarette smoking on the fasted gallbladder volume in man. *Ital J Gastroenterol* 1989; **21**: 337-339
24 **Seino Y**, Tsuda K, Mori K, Takemura J, Kuzuya H, Yamamura T, Kotoura Y, Ito N, Imura H. Effect of smoking on gastrointestinal hormone secretion. In: Proceedings on the 5th world conference, smoking and health. Winnipeg, Canada: Canadian Council on Smoking and Health, 1983: 207-212
25 **Conter R**, Roslyn JJ, Muller EL, Pitt HA, Porter-Fink V, Den-Besten L, Taylor IL. Effect of pancreatic polypeptide on gallbladder filling. *J Surg Res* 1985; **38**: 461-467
26 **Forgacs IC**, Maisey MN, Murphy GM, Dowling RH. Influence of gallstones and ursodeoxycholic acid therapy on gallbladder emptying. *Gastroenterology* 1984; **87**: 299-307
27 **Admirand WH**, Small DM. The physicochemical basis of cholesterol gallstone formation in man. *J Clin Invest* 1968; **47**: 1043-1052
28 **Baxter JN**. Gall-bladder emptying. *J Gastroenterol Hepatol* 1989; **4**: 353-372
29 **Behar J**, Lee KY, Thompson WR, Biancani P. Gallbladder contraction in patients with pigment and cholesterol stones. *Gastroenterology* 1989; **97**: 1479-1484
30 **Fisher RS**, Rock E, Malmud LS. Cholinergic effects on gallbladder emptying in humans. *Gastroenterology* 1985; **89**: 716-722
31 **Dillon L**, Glenn F, Becker CG. Induction of acalculous cholecystitis and pneumonitis in dogs following inhalation of constituents of cigarette smoke condensate. *Am J Pathol* 1982; **109**: 253-258
32 **Parkman HP**, James AN, Bogar LJ, Bartula LL, Thomas RM, Ryan JP, Myers SI. Effect of acalculous cholecystitis on gallbladder neuromuscular transmission and contractility. *J Surg Res* 2000; **88**: 186-192

S- Editor Wang J **L- Editor** Wang XL **E- Editor** Bai SH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5544-5549
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Furazolidone-based triple therapy for *H pylori* gastritis in children

Elisabete Kawakami, Rodrigo Strehl Machado, Silvio Kazuo Ogata, Marini Langner, Erika Fukushima, Anna Paula Carelli, Vania Cláudia Guimarães Bonucci, Francy Reis Silva Patrício

Elisabete Kawakami, Rodrigo Strehl Machado, Silvio Kazuo Ogata, Marini Langner, Erika Fukushima, Anna Paula Carelli, Vania Cláudia Guimarães Bonucci, Peptic Diseases Outpatient Clinic, Pediatric Gastroenterology Division, Department of Pediatric, Universidade Federal de São Paulo/Escola Paulista de Medicina, São Paulo, SP, Brazil
Francy Reis Silva Patrício, Department of Pathology, Universidade Federal de São Paulo/Escola Paulista de Medicina, São Paulo, SP, Brazil
Correspondence to: Rodrigo Strehl Machado, 441 Pedro de Toledo Street, Sao Paulo 04041-039, Brazil. rodrigo@gastroped.epm.br
Telephone: +55-11-55794351 Fax: +55-11-55795834
Received: 2006- 02-15 Accepted: 2006-04-21

Abstract

AIM: To evaluate the furazolidone-based triple therapy in children with symptomatic *H pylori* gastritis.

METHODS: A prospective and consecutive open trial was carried out. The study included 38 patients with upper digestive symptoms sufficiently severe to warrant endoscopic investigation. *H pylori* status was defined based both on histology and on positive ^{13}C-urea breath test. Drug regimen was a seven-day course of omeprazole, clarithromycin and furazolidone (100 mg, 200 mg if over 30 kg) twice daily. Eradication of *H pylori* was assessed two months after treatment by histology and ^{13}C-urea breath test. Further clinical evaluation was performed 7 d, 2 and 6 mo after the treatment.

RESULTS: Thirty-eight patients (24 females, 14 males) were included. Their age ranged from 4 to 17.8 (mean 10.9 ± 3.7) years. On intent-to-treat analysis (n = 38), the eradication rate of *H pylori* was 73.7% (95% CI, 65.2%-82%) whereas in per-protocol analysis (n = 33) it was 84.8% (95% CI, 78.5%-91%). All the patients with duodenal ulcer (n = 7) were successfully treated (100% *vs* 56.2% with antral nodularity). Side effects were reported in 26 patients (68.4%), mainly vomiting (14/26) and abdominal pain (n = 13). Successfully treated dyspeptic patients showed improvement in 78.9% of *H pylori*-negative patients after six months and in 50% of *H pylori*-positive patients after six months of treatment.

CONCLUSION: Triple therapy with furazolidone achieves moderate efficacy in *H pylori* treatment. The eradication rate seems to be higher in patients with duodenal ulcer.

Key words: Furazolidone; *H pylori* treatment; Gastritis; Children

Kawakami E, Machado RS, Ogata SK, Langner M, Fukushima E, Carelli AP, Bonucci VCG, Patrício FRS. Furazolidone-based triple therapy for *H pylori* gastritis in children. *World J Gastroenterol* 2006; 12(34): 5544-5549

http://www.wjgnet.com/1007-9327/12/5544.asp

INTRODUCTION

Available treatment regimens for gastritis due to *H pylori* show a lower success rate in children than in adult patients in the same geographic region. Several factors influence *H pylori* eradication rate, such as compliance with treatment, mutations generating resistances, sanctuaries (sites where there is no contact between the bacterium and antimicrobial drugs), deficiency in immunity of the host, low gastric pH, large infecting load, dormant forms and reinfection[1]. *H pylori* treatment in children has specific difficulties and the success rate of the current options is worse than that in adults. It is speculated that in children there is less compliance with treatment and the prevalence of resistant strains is higher due to the greater exposure to antibiotics because of common childhood diseases. In addition, patients without peptic ulcer seem to present a lower success rate[2].

The ideal treatment regimen for *H pylori* eradication should present a higher than 80% cure index on intention-to-treat analysis. Antimicrobial resistance is a major concern and in the past decade there was the emergence of clarithromycin-resistant strains, reaching 34.7% of isolates in some, mainly developed countries[3-5]. Resistance occurs due to punctual mutations in the 23S rRNA[5]. Nevertheless, in developing countries, resistance to metronidazole is highly prevalent, possibly due to overuse of this antimicrobial drug in gynecology and parasite treatment; thus this drug is not a viable alternative[6]. Although amoxicillin resistance is regarded a rare phenomenon, recently there are an increasing number of

reports on resistant strains[7].

Furazolidone emerges as an alternative for therapeutic regimens in developing countries due to its low cost and prevalence of resistant strains. This antimicrobial is a monoamine oxidase inhibitor usually utilized in the treatment of giardiasis. There are studies demonstrating its efficacy and safety in several developing countrie[8-12]. The drug has been used in *H pylori* treatment regimens since 1990, initially tested in China with a reasonable success rate and constitutes an alternative in situations where there is high resistance prevalence to nitroimidazoles. In our country a triple regimen with furazolidone, clarithromycin and omeprazole can attain a 90% cure in adult patients on intention-to-treat analysis[13] while with furazolidone, levofloxacin and rabeprazole can reach 83% eradication as a third-line regimen[14]. Consensus statements providing guidelines for the management of *H pylori* infection in children have made recommendations for therapy based on data derived from adult trials but have not provided suggestions on therapeutic options[15,16]. The present study was to evaluate the triple regimen with omeprazole, clarithromycin and furazolidone for 7 d in children with *H pylori* gastritis, being the first study in children.

MATERIALS AND METHODS

Subjects

To warrant *H pylori* eradication, patients meeting the following criteria were included: (1) duodenal ulcer or erosive duodenitis (n = 7); (2) ulcer-like functional dyspepsia, according to the Rome II criteria, sufficiently severe to justify upper gastrointestinal endoscopy and without major mucosal abnormalities (n = 29)[17]; (3) upper gastrointestinal bleeding (n = 1); (4) iron-deficiency anemia refractory to standard treatment (n = 1). Patients with former unsuccessful treatment for *H pylori* or with other organic diseases that could explain the symptoms were excluded. The study was approved by the Ethics Committee of the "Universidade Federal de São Paulo/ Escola Paulista de Medicina". On inclusion of the patients in the study, the responsible person(s) received written information about the patients and signed a free and informed consent.

Diagnosis of infection

Endoscopic examination was performed by our team, under deep sedation or general anesthesia supervised by an anesthetist. Four biopsy specimens were collected from the gastric antrum at approximately 2 cm from the pylorus, two for rapid urease test and two for histological analysis. The latter two were fixed in 100 mL/L formol, placed on filter paper and stained with hematoxylin-eosin and modified Giemsa. The findings were described according to modified Sydney criteria[18]. The histological diagnosis of the infection was established by an experienced pathologist, based on the typical appearance of the bacterium along the mucus layer covering the gastric mucous membrane. Rapid urease test was performed with a non-commercial solution (100 mg/mL aqueous urea solution with 10 mg/ mL phenol red) as previously described[19]. The patient was considered infected when both tests were positive and non-infected when both were negative.

Table 1 Endoscopic findings

Endoscopic findings	*n*	%
Nodular gastritis	16	42.1
Normal	13	34.2
Duodenal ulcer or erosive bulbitis	4	10.5
Erosive duodenitis and nodular gastritis	1	2.6
Erosive gastritis	1	2.6
Nodular gastritis and esophagitis	1	2.6
Duodenal ulcer and esophagitis	1	2.6
Erosive duodenitis, esophagitis and nodular gastritis	1	2.6

H pylori treatment

The triple regimen was administered twice daily for seven days: 100 mg furazolidone or 200 mg furazolidone (> 30 kg), 250 mg clarithromycinor or 500 mg clarithromycin (> 30 kg), 10 mg omeprazole or 20 mg omeprazole (> 30 kg). Antibiotics were prescribed after meals whereas omeprazole was adminstered before the first meal. Patients and their responsible persons were advised to maintain the treatment even with minor adverse effects. On the last day of the treatment, a complete physical examination was performed to evaluate the clinical conditions of patients. During this examination the patients were asked about adverse effects, and compliance was controlled by return of empty medication blisters. Compliance with treatment was defined by over 75% intake of the prescribed doses.

H pylori eradication

A renewed clinical and endoscopic evaluation was performed two months after the treatment, with collection of antrum and corpus biopsies for histology and rapid urease test. Patients whose responsible persons did not give consent to another endoscopy were evaluated using the ^{13}C-urea breath test. This test was performed as previously described[20]. The cutoff value of breath test was delta over baseline 4‰. The patients were submitted to a new clinical evaluation two and six months after treatment and asked about the progress of symptoms and the frequency and intensity of epigastric pain in those with dyspepsia.

Statistical analysis

Continuous variables were expressed by calculation of the mean and standard deviation. The eradication rates were expressed by calculation of the proportion with an 85% confidence interval (95% CI). Treatment groups were compared using Pearson's chi-square test with Fisher's exact test when necessary. Factors associated with treatment success were evaluated by estimation of the odds ratio with 95% confidence interval. $P < 0.05$ was considered statistically significant.

RESULTS

Thirty-eight patients were included (24 females) with their

Table 2 Adverse effects reported by 26 of the 38 patients

Adverse effect	*n*	%
Vomiting	14	36.8
Abdominal pain	13	34.2
Metallic taste	6	15.8
Diarrhea	5	13.2
Nausea	5	13.2
Dizziness	2	5.3
Headache	2	5.3
Asthenia	1	2.6
Skin rash	1	2.6

age ranging from 4 to 17.8 (mean 10.9 ± 3.7) years. Results of the endoscopic examinations are shown in Table 1. The histological analysis showed active chronic gastritis in all patients. Intensity of the neutrophil infiltrate was low in 9 patients (23.7%), moderate in 19 (50%) and intense in 10 (26.3%). Intensity of bacterial density on histology was low in 11 patients (28.9%), moderate in 19 (50%) and intense in 8 (21.1%).

Slight side effects were reported in 26 patients (68.4%), disappearing with the interruption of the treatment (Table 2). Compliance with the protocol occurred in 33/38 patients (86.6%), intake of medications was not correct in 4 patients and control of treatment was very late in one. The eradication rate of infection was 84.8% in 28/33 patients treated according to the protocol (95% CI: 78.5%-91%), and 73.7% by intent-to-treat analysis in 28/38 patients (95% CI: 65.4%-82%). Influence of demographic, clinical and histologic data on the success of treatment is shown in Table 3. The infection was eradicated in all the 7 patients with erosive duodenitis or duodenal ulcer, while only 9/16 (56.2%) patients with nodular antrum gastritis as a single alteration were successfully treated ($P = 0.08$).

Evaluation of *H pylori* eradication was performed through histology and ^{13}C-urea breath test in 26 patients who were successfully treated and breath test in 2 patients who were successfully treated. *H pylori* eradication evaluation was not performed in 4 patients whose medication intake was less than 25% of that prescribed. Results of endoscopy were normal in 21 (65.6%), nodular antrum gastritis in 9 (28.1%) and erosive gastritis in 1 (6.5%). Cure of infection was achieved in two patients with erosive gastritis. After the treatment, among the 26 cured patients, 9 (34.6%) had normal histology, 7 (26.9%) inactive chronic gastritis, 9 (34.6%) low neutrophil infiltrate and 1 (3.8%) moderate neutrophil infiltrate. Among the 6 patients remaining infected, a second endoscopy revealed low neutrophil infiltrate in 4 (67%) and moderate neutrophil infiltrate in 2 (33%), decreased neutrophil infiltrate in 3 (50%). Gastritis activity did not worsen in any of the patients.

Clinical progress

Success treatment of *H pylori* infection in patients with functional dyspepsia is shown in Figure 1. After two months of treatment, 63% of the eradicated dyspeptic patients and 60% of the non-eradicated patients reported improvement of symptoms ($P = 1$), while at 6-mo follow-up 78.9% successfully treated patients and 50% of the non-eradicated patients reported improvement of symptoms ($P = 0.2$). The hematological parameters of the patients with refractory iron deficiency anemia returned to normal after treatment and all patients with duodenal ulcer or erosive duodenitis were asymptomatic.

Table 3 Influence of clinical and histological variables on therapeutic success

	Eradication rate (%)	Odds ratio[1]	95% CI	*P*
Demographic data				
Female gender	66.7	0.33	0.03-2.18	0.27
Age ⩽ 10 yr	66.7	0.56	0.1-3.11	0.47
Indication for treatment				
Ulcer-like functional dyspepsia	65.5	0.00	0.0-1.23	0.08
Initial endoscopy				
Normal examination	76.9	1.30	0.23-9.44	1
Nodular gastritis[2]	56.2	0.24	0.03-1.39	0.08
Duodenal ulcer or erosive bulbitis	100	undefined	undefined	0.16
Histology				
Intense activity	70.0	0.70	0.13-5.97	1
Moderate activity	78.9	1.73	0.32-10.17	0.46
Light activity	66.7	0.67	0.10-5.28	0.68
Intense density	75.0	1.09	0.15-13.19	1
Moderate density	73.7	1.00	0.18-5.45	1
Light density	72.7	0.93	0.16-7.01	1
Adverse effects				
Yes	76.9	1.67	0.27-9.37	0.69

[1] for therapeutic success; [2] without duodenal ulcer or erosive duodenitis.

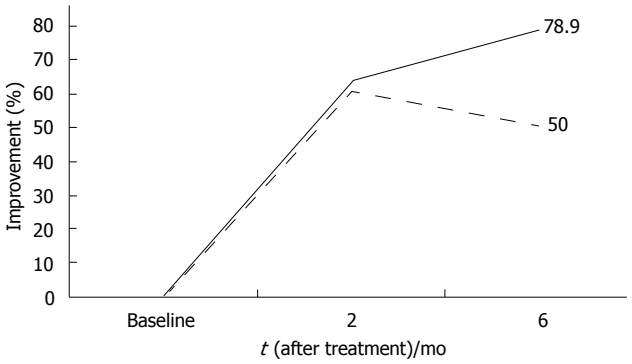


Figure 1 Patients with non-ulcer dyspepsia reporting symptom improvement after treatment according to *H pylori* infection eradication in 2- and 6-mo follow-up. Continuous line: successfully eradicated patients; Dashed line: not eradicated patients. *P* (2 mo) = 1, *P* (6 mo) = 0.2.

DISCUSSION

The attained success rate (73.3% by intent-to-treat analysis) was higher than that observed by a former study with amoxicillin, clarithromycin and omeprazole for 7 d (50%, 95% CI: 19%-81%) in our service, but similar to that for 10 d (73%, 95% CI: 51%-95%) [21]. There is no other study

comparing triple therapy for 7 d with a longer treatment period. Other studies with clarithromycin, amoxicillin and proton pump inhibitor reported that the eradication rate of *H pylori* is 54%-77.8% in children[6,22,23]. At present, the first-line regimen recommended by Brazilian consensus for the treatment of adults is the triple treatment with clarithromycin, amoxicillin (or furazolidone) and proton pump inhibitor for 7-14 d[24]. A seven-day treatment period was chosen in our study because it is as effective as a ten-day period. The efficiency of a longer treatment period (14 d) is only 9% higher with a significant cost increase[25]. In Brazil there are 16% clarithromycin-resistant and 55% metronidazole-resistant strains, thus requiring alternative regimens for classical compositions[25,26]. Recently a sequential therapy has been described, consisting of two treatment regimes for five consecutive days. In these studies, amoxicillin and proton pump inhibitor (PPI) are used for five days followed by PPI, clarithromycin and tinidazole for another five days[27]. In children the regimen is more efficient than the traditional treatment with amoxicillin, metronidazole and PPI for 10 d (97.3%, 95% CI: 86.2%-99.5% *vs* 75.5%, 95% CI: 59.8%-86.7%), and has no more side effects (global rate 12%)[28]. A sequential regimen exposes the patients to three different drug classes as a first line treatment, which may make the choice of a second-line treatment difficult in eventual therapeutic failures.

There are no clinical or laboratory factors associated with a better result of the treatment (Table 3). The eradication rate of *H pylori* observed in our study in patients with duodenal ulcer is similar to that observed by Dani and coworkers[13] in adults with ulcer disease, but the difference in the eradication rate of *H pylori* in patients with functional non-ulcer dyspepsia did not reach statistical significance, perhaps due to the small number of patients with ulcer included in our study ($P = 0.08$). A recent study has shown a lower eradication rate of *H pylori* in patients with non-ulcer dyspepsia[2]. Justifying factors include clarithromycin susceptibility to strains in patients with dyspepsia, less strain virulence (CagA negative) and differences in compliance with treatment. The lowest eradication index of *H pylori* observed in children may be due to the low prevalence of duodenal ulcer[29]. On the other hand, patients with antral nodularity present a lower eradication rate of *H pylori* (56.2%), but lymphoid follicles are found to be associated with treatment failure in adult patients[30]. Antral nodularity may be related to a higher inflammation intensity and more aggressive strains. However, it seems more difficult to eradicate infection with a CagA negative strain[31]. The high incidence of side effects (67.6%), although slight and self-limited, constitutes an inconvenience for the studied regimen. The reported side effects are slight and do not compromise the success treatment (Table 3). The reported symptoms may be attributed to clarithromycin or to furazolidone. Furazolidone is a nitrofuran compound which has been used in the treatment of giardiasis since the 1950s. The drug has minimal adverse effects, mostly nausea, vomiting and diarrhea. Other side effects include brown discoloration of urine and hemolysis in glucose-6-phosphate dehydrogenase deficient patients and infants younger than 1-year old[32]. Treatment regimens with furazolidone usually present a higher incidence of side effects than traditional alternatives[9]. Lower furazolidone doses neither affect the success treatment rate, nor decrease the frequency of adverse effects[11].

The omeprazole dose used may be considered small in view of recent evidence that some patients need higher doses[33]. The importance of antisecretory drugs in the eradication regimen is their direct effect on the bacterium and the better antibiotic activity at high pH[1]. Cytochrome P2C19 is responsible for hepatic metabolism of some proton pump inhibitors, such as omeprazole, and the CYP2C19 genotype, an isoform, is associated with more rapid metabolism, constituting another risk factor for unsuccessful eradication treatment of *H pylori*[34]. However, there are no studies describing the prevalent genotypes. Finally, some of our patients used generic omeprazole. The efficacy of *H pylori* eradication regimen with generic medication is lower than that with proprietary drugs in adult patients in Russia[35]. Omeprazole bioavailability depends on its presentation

Most of our patients presented non-ulcer dyspepsia, a situation in which treatment of *H pylori* infection is still controversial. The treatment seems to be beneficial to some adult patients and it is estimated that 1 in 18 patients improves after the treatment[36]. There are still important limitations in therapeutic trials for dyspepsia in children. There are no criteria for the selection of patients and no validated diagnostic and functional dyspepsia severity scores in children, which makes the generalization of results difficult. Over 50% of physicians in USA treat *H pylori* in children with dyspeptic symptoms without endoscopy[37]. In spite of the higher symptom improvement proportion among the successfully treated patients (78.9% *vs* 50%), the study could not draw a conclusion about the clinical validity of the treatment because of the small number of studied patients (Figure 1). Other studies have reported a similar response rate in children with recurrent chronic abdominal pain[6,38]. Long-term symptom resolution in patients with severe symptoms requiring endoscopy shows differences in epigastric pain resolution between *H pylori*-negative (3/26) and positive (7/10) patients ($P = 0.001$) after one to two years[38]. Early clinical evaluation may underestimate the beneficial effects of the treatment and longer follow-up periods may show effective *H pylori* eradication and symptom resolution.

The tested regimen may be superior to the regimen with clarithromycin, amoxicillin and omeprazole, and can be used in the treatment of infection in patients with duodenal ulcer. Its success rate is lower in non-ulcer dyspepsia. Treatment regimens with a longer time should be tested in children.

REFERENCES

1 **Mégraud F**, Lamouliatte H. Review article: the treatment of refractory *Helicobacter pylori* infection. *Aliment Pharmacol Ther* 2003; **17**: 1333-1343

2 **Wong WM**, Xiao SD, Hu PJ, Wang WH, Gu Q, Huang JQ, Xia HH, Wu SM, Li CJ, Chen MH, Cui Y, Lai KC, Hu WH, Chan CK, Lam SK, Wong BC. Standard treatment for *Helicobacter pylori* infection is suboptimal in non-ulcer dyspepsia compared

with duodenal ulcer in Chinese. *Aliment Pharmacol Ther* 2005; **21**: 73-81
3 **Perez Aldana L**, Kato M, Nakagawa S, Kawarasaki M, Nagasako T, Mizushima T, Oda H, Kodaira J, Shimizu Y, Komatsu Y, Zheng R, Takeda H, Sugiyama T, Asaka M. The relationship between consumption of antimicrobial agents and the prevalence of primary *Helicobacter pylori* resistance. *Helicobacter* 2002; **7**: 306-309
4 **Kato S**, Fujimura S, Udagawa H, Shimizu T, Maisawa S, Ozawa K, Iinuma K. Antibiotic resistance of *Helicobacter pylori* strains in Japanese children. *J Clin Microbiol* 2002; **40**: 649-653
5 **Larrosa-Haro A**, Martínez-Puente EO, Coello-Ramírez P, Castillo de León YA, Bojórquez-Ramos Mdel C, Macías-Rosales R, García-Salazar O, Vázquez-Camacho G, Flores Márquez MR. [Efficacy of two *Helicobacter pylori* eradication treatments in children with recurrent abdominal pain]. *Rev Gastroenterol Mex* 2004; **69**: 76-82
6 **Watanabe K**, Tanaka A, Imase K, Tokunaga K, Sugano H, Kai A, Ishida H, Itoh T, Takahashi S. Amoxicillin resistance in *Helicobacter pylori*: studies from Tokyo, Japan from 1985 to 2003. *Helicobacter* 2005; **10**: 4-11
7 **Lu H**, Zhang DZ, Hu PJ, Li ZS, Lu XH, Fang XC, Xiao SD. One-week regimens containing ranitidine bismuth citrate, furazolidone and either amoxicillin or tetracycline effectively eradicate *Helicobacter pylori*: a multicentre, randomized, double-blind study. *Aliment Pharmacol Ther* 2001; **15**: 1975-1979
8 **Xiao SD**, Liu WZ, Hu PJ, Ouyang Q, Wang JL, Zhou LY, Cheng NN. A multicentre study on eradication of *Helicobacter pylori* using four 1-week triple therapies in China. *Aliment Pharmacol Ther* 2001; **15**: 81-86
9 **Araujo Castillo R**, Pinto Valdivia JL, Ramírez D, Cok García J, Bussalleu Rivera A. [New ultrashort scheme for helicobacter pylori infection eradication using tetracyline, furazolidone and colloidal bismuth subcitrate in dyspeptic patients with or without peptic ulceration in the National Hospital Cayetano Heredia]. *Rev Gastroenterol Peru* 2005; **25**: 23-41
10 **Roghani HS**, Massarrat S, Shirekhoda M, Butorab Z. Effect of different doses of furazolidone with amoxicillin and omeprazole on eradication of *Helicobacter pylori*. *J Gastroenterol Hepatol* 2003; **18**: 778-782
11 **Fakheri H**, Merat S, Hosseini V, Malekzadeh R. Low-dose furazolidone in triple and quadruple regimens for *Helicobacter pylori* eradication. *Aliment Pharmacol Ther* 2004; **19**: 89-93
12 **Dani R**, Queiroz DM, Dias MG, Franco JM, Magalhães LC, Mendes GS, Moreira LS, De Castro LP, Toppa NH, Rocha GA, Cabral MM, Salles PG. Omeprazole, clarithromycin and furazolidone for the eradication of *Helicobacter pylori* in patients with duodenal ulcer. *Aliment Pharmacol Ther* 1999; **13**: 1647-1652
13 **Coelho LG**, Moretzsohn LD, Vieira WL, Gallo MA, Passos MC, Cindr JM, Cerqueira MC, Vitiello L, Ribeiro ML, Mendonça S, Pedrazzoli-Júnior J, Castro LP. New once-daily, highly effective rescue triple therapy after multiple *Helicobacter pylori* treatment failures: a pilot study. *Aliment Pharmacol Ther* 2005; **21**: 783-787
14 **Drumm B**, Koletzko S, Oderda G. Helicobacter pylori infection in children: a consensus statement. European Paediatric Task Force on Helicobacter pylori. *J Pediatr Gastroenterol Nutr* 2000; **30**: 207-213
15 **Gold BD**, Colletti RB, Abbott M, Czinn SJ, Elitsur Y, Hassall E, Macarthur C, Snyder J, Sherman PM. Helicobacter pylori infection in children: recommendations for diagnosis and treatment. *J Pediatr Gastroenterol Nutr* 2000; **31**: 490-497
16 **Rasquin-Weber A**, Hyman PE, Cucchiara S, Fleisher DR, Hyams JS, Milla PJ, Staiano A. Childhood functional gastrointestinal disorders. *Gut* 1999; **45** Suppl 2: II60-II68
17 **Dixon MF**, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996; **20**: 1161-1181
18 **Ogata SK**, Kawakami E, Patrício FR, Pedroso MZ, Santos AM. Evaluation of invasive and non-invasive methods for the diagnosis of *Helicobacter pylori* infection in symptomatic children and adolescents. *Sao Paulo Med J* 2001; **119**: 67-71
19 **Machado RS**, Patrício FR, Kawakami E. 13C-urea breath test to diagnose *Helicobacter pylori* infection in children aged up to 6 years. *Helicobacter* 2004; **9**: 39-45
20 **Kawakami E**, Ogata SK, Portorreal AC, Magni AM, Pardo ML, Patrício FR. Triple therapy with clarithromycin, amoxicillin and omeprazole for *Helicobacter pylori* eradication in children and adolescents. *Arq Gastroenterol* 2001; **38:** 203-206
21 **Gottrand F**, Kalach N, Spyckerelle C, Guimber D, Mougenot JF, Tounian P, Lenaerts C, Roquelaure B, Lachaux A, Morali A, Dupont C, Maurage C, Husson MO, Barthélemy P. Omeprazole combined with amoxicillin and clarithromycin in the eradication of *Helicobacter pylori* in children with gastritis: A prospective randomized double-blind trial. *J Pediatr* 2001; **139**: 664-668
22 **Koçak N,** Saltik IN, Ozen H, Yüce A, Gürakan F. Lansoprazole triple therapy for Turkish children with *Helicobacter pylori* infections. *J Pediatr Gastroenterol Nutr* 2001; **32**: 614
23 **Coelho LG**, Zaterka S. [Second Brazilian Consensus Conference on Helicobacter pylori infection]. *Arq Gastroenterol* 2005; **42**: 128-132
24 **Bytzer P**, O'Morain C. Treatment of Helicobacter pylori. *Helicobacter* 2005; **10** Suppl 1: 40-46
25 **Godoy AP**, Ribeiro ML, Benvengo YH, Vitiello L, Miranda Mde C, Mendonça S, Pedrazzoli J Jr. Analysis of antimicrobial susceptibility and virulence factors in *Helicobacter pylori* clinical isolates. *BMC Gastroenterol* 2003; **3**: 20
26 **De Francesco V**, Della Valle N, Stoppino V, Amoruso A, Muscatiello N, Panella C, Ierardi E. Effectiveness and pharmaceutical cost of sequential treatment for *Helicobacter pylori* in patients with non-ulcer dyspepsia. *Aliment Pharmacol Ther* 2004; **19**: 993-998
27 **Francavilla R**, Lionetti E, Castellaneta SP, Magistà AM, Boscarelli G, Piscitelli D, Amoruso A, Di Leo A, Miniello VL, Francavilla A, Cavallo L, Ierardi E. Improved efficacy of 10-Day sequential treatment for Helicobacter pylori eradication in children: a randomized trial. *Gastroenterology* 2005; **129**: 1414-1419
28 **Oderda G**, Rapa A, Bona G. A systematic review of Helicobacter pylori eradication treatment schedules in children. *Aliment Pharmacol Ther* 2000; **14** Suppl 3: 59-66
29 **Georgopoulos SD**, Ladas SD, Karatapanis S, Mentis A, Spiliadi C, Artikis V, Raptis SA. Factors that may affect treatment outcome of triple *Helicobacter pylori* eradication therapy with omeprazole, amoxicillin, and clarithromycin. *Dig Dis Sci* 2000; **45**: 63-67
30 **Queiroz DM**, Dani R, Silva LD, Santos A, Moreira LS, Rocha GA, Corrêa PR, Reis LF, Nogueira AM, Alvares Cabral MM, Esteves AM, Tanure J. Factors associated with treatment failure of *Helicobacter pylori* infection in a developing country. *J Clin Gastroenterol* 2002; **35**: 315-320
31 **Gardner TB**, Hill DR. Treatment of giardiasis. *Clin Microbiol Rev* 2001; **14:** 114-128
32 **Miehlke S**, Mannes GA, Lehn N, Hele C, Stolte M, Bayerdörffer E. An increasing dose of omeprazole combined with amoxycillin cures *Helicobacter pylori* infection more effectively. *Aliment Pharmacol Ther* 1997; **11**: 323-329
33 **Take S**, Mizuno M, Ishiki K, Nagahara Y, Yoshida T, Inaba T, Yamamoto K, Okada H, Yokota K, Oguma K, Shiratori Y. Interleukin-1beta genetic polymorphism influences the effect of cytochrome P 2C19 genotype on the cure rate of 1-week triple therapy for *Helicobacter pylori* infection. *Am J Gastroenterol* 2003; **98**: 2403-2408
34 **Shcherbakov PL**, Filin VA, Volkov IA, Tatarinov PA, Belousov YB. A randomized comparison of triple therapy *Helicobacter pylori* eradication regimens in children with peptic ulcers. *J Int Med Res* 2001; **29**: 147-153
35 **Moayyedi P**, Soo S, Deeks J, Delaney B, Harris A, Innes M, Oakes R, Wilson S, Roalfe A, Bennett C, Forman D. Eradication of *Helicobacter pylori* for non-ulcer dyspepsia. *Cochrane Database Syst Rev* 2005; CD002096
36 **Chang HY**, Sharma VK, Howden CW, Gold BD. Knowledge, attitudes, and practice styles of North American pediatric gas-

troenterologists: *Helicobacter pylori* infection. *J Pediatr Gastroenterol Nutr* 2003; **36**: 235-240

37 **Ozçay F,** Koçak N, Temizel IN, Demir H, Ozen H, Yüce A, Gürakan F. *Helicobacter pylori* infection in Turkish children: comparison of diagnostic tests, evaluation of eradication rate, and changes in symptoms after eradication. *Helicobacter* 2004; **9:** 242-248

38 **Oderda G**, Marinello D, Lerro P, Kuvidi M, de'Angelis GL, Ferzetti A, Cucchiara S, Franco MT, Romano C, Strisciuglio P, Pensabene L. Dual vs. triple therapy for childhood *Helicobacter pylori* gastritis: a double-blind randomized multicentre trial. *Helicobacter* 2004; **9**: 293-301

S- Editor Pan BR **L- Editor** Wang XL **E- Editor** Bai SH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5550-5553
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Prevalence of SLC22A4, SLC22A5 and CARD15 gene mutations in Hungarian pediatric patients with Crohn's disease

Judit Bene, Lili Magyari, Gábor Talián, Katalin Komlósi, Beáta Gasztonyi, Beáta Tari, Ágnes Várkonyi, Gyula Mózsik, Béla Melegh

Judit Bene, MTA PTE Clinical Genetics Research Group of Hungarian Academy of Sciences at the University of Pécs, Pécs, Hungary
Judit Bene, Lili Magyari, Gábor Talián, Katalin Komlósi, Béla Melegh, Department of Medical Genetics and Child Development, University of Pécs, Pécs, Hungary
Beáta Tari, Ágnes Várkonyi, Department of Pediatrics and Child Health Center, University of Szeged, Szeged, Hungary
Beáta Gasztonyi, Gyula Mózsik, 1st Department of Medicine, School of Medicine, University of Pécs, Pécs, Hungary
Supported by the grant of Hungarian Science Foundation No. OTKA T 49589
Correspondence to: Dr. Béla Melegh, Professor of Medical Genetics and Pediatrics, Department of Medical Genetics and Child Development, University of Pécs, H-7624 Pécs, Szigeti 12, Hungary. bela.melegh@aok.pte.hu
Telephone: +36-72-536427 Fax: +36-72-536427
Received: 2006-06-13 Accepted: 2006-07-07

Abstract

AIM: To investigate the frequency of the common NOD2/CARD15 susceptibility variants and two functional polymorphisms of OCTN cation transporter genes in Hungarian pediatric patients with Crohn's disease (CD).

METHODS: A cohort of 19 unrelated pediatric and 55 unrelated adult patients with Crohn's disease and 49 healthy controls were studied. Genotyping of the three common CD-associated CARD15 variants (Arg702Trp, Gly908Arg and 1007finsC changes) with the SLC22A4 1672C→T, and SLC22A5 -207G→C mutations was performed by direct sequencing of the specific regions of these genes.

RESULTS: At least one CARD15 mutation was present in 52.6% of the children and in 34.5% of the adults compared to 14.3% in controls. Surprisingly, strongly different mutation profile was detected in the pediatric *versus* adult patients. While the G908R and 1007finsC variants were 18.4% and 21.1% in the pediatric group, they were 1.82% and 11.8% in the adults, and were 1.02% and 3.06% in the controls, respectively. The R702W allele was increased approximately two-fold in the adult subjects, while in the pediatric group it was only approximately 64% of the controls (9.09% in the adults, 2.63% in pediatric patients, and 4.08% in the controls). No accumulation of the OCTN variants was observed in any patient group *versus* the controls.

CONCLUSION: The frequency of the NOD2/CARD15 susceptibility variants in the Hungarian pediatric CD population is high and the profile differs from the adult CD patients, whereas the results for SLC22A4 and SLC22A5 mutation screening do not confirm the assumption that the carriage of these genotypes means an obligatory susceptibility to CD.

Key words: OCTN1; OCTN2; NOD2/CARD15; Crohn's disease

Bene J, Magyari L, Talián G, Komlósi K, Gasztonyi B, Tari B, Várkonyi Á, Mózsik G, Melegh B. Prevalence of SLC22A4, SLC22A5 and CARD15 gene mutations in Hungarian pediatric patients with Crohn's disease. *World J Gastroenterol* 2006; 12(34): 5550-5553

http://www.wjgnet.com/1007-9327/12/5550.asp

INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract. Although the peak onset of the disease typically occurs in the second and third decades of life[1], the incidence of pediatric cases has been strongly increasing recently[2]. Despite the comprehensive research that has been made to discover the background of the disease, the etiology is still unknown. Besides the environmental effects, it is supposed that genetic susceptibility plays a crucial role in the development of the disease[3,4].

Genome-wide linkage analyses have resulted in identification of several loci of potential CD susceptibility genes[5-11]. CARD15 (NOD2) gene, which is located at the pericentromeric region of chromosome 16, was the first gene to be identified as CD gene[12-14]. NOD2 is an intracellular protein expressed in peripheral blood monocytes, Paneth and intestinal epithelial cells; and it is important for inflammatory signal transduction *via* activation of the transcription factor, nuclear factor kappa-B (NF-κB)[15]. Several studies on Caucasian populations have reported an association between CARD mutations and CD. Three coding variants (R702W, G908R

and 1007finsC) have been identified as independent risk factors for development of CD.

Recently two polymorphisms in the carnitine/organic cation transporter gene cluster (SLC22A4 and SLC22A5, encoding OCTN1 and OCTN2, respectively) have been found to confer risk for CD[16]. The aim of the present study was to investigate the prevalence of these two functional variants of the SLC22A4 and SLC22A5 genes and the three CARD15 mutations in Hungarian pediatric population with CD.

MATERIALS AND METHODS

Patients

We examined 19 pediatric (14 males and 5 females; mean age: 13.4 years) and 55 adult (27 male and 28 female with real maturity onset disease; mean age: 42.3 years) patients with CD. This cohort was compared with 49 age- and sex-matched healthy controls (28 males and 21 females; mean age: 14.4 years). Both the pediatric and adult CD patients exhibited different clinical manifestations, therefore they represented mixed clinical CD populations. The diagnosis was confirmed by clinical, radiological, endoscopic and histological findings. Informed consent was obtained from each participant of the study and the study design was approved by the Local Ethics Committee.

Methods

Genomic DNA from the patients and the controls was isolated from peripheral blood using standard desalting procedure.

The presence of the NOD2 and OCTN variants was detected by direct sequencing using the primers designed in our laboratory. The primers' sequences for the PCR amplification as well as for the sequencing and annealing temperatures are listed in Table 1. The PCR was carried out in a final volume of 50 μL containing 200 μmol/L of each dNTP, 2 units of Taq polymerase, 5 μL of reaction buffer [100 mmol/L Tris HCl (pH 9.0), 500 mmol/L KCl, 15 mmol/L $MgCl_2$, 0.2 μmol/L of each primer and 1 μg of DNA to be amplified. The amplification was performed for a total of 35 cycles in an MJ Research PTC-200 thermal cycler. The amplification conditions were: pre-denaturation at 95℃ for 2 min, denaturation at 95℃ for 30 s, annealing for 30 s at the temperatures listed in Table 1 for the different SNP, primer extension at 72℃ for 30 s, and the final extension at 72℃ for 5 min. For DNA sequencing, a BigDye Terminator labeling was used and the analysis was performed in an ABI 3100 automatic sequencer.

Statistical analysis

Chi-square test (cross-table analysis) was used to analyze the possible associations with mutations in comparison of either the susceptibility variants and/or the normal haplotypes. $P < 0.05$ was considered statistically significant.

RESULTS

The allele frequencies are shown in Table 2. A total of 52.6% of pediatric patients with Crohn's disease carried at least one NOD2 mutation compared to 34.5% of adult patients and to 14.3% of the controls (pediatric patients *vs* controls $P < 0.05$).

While the T allele frequency, leading to heterozygous and homozygous R702W mutation, was increased approximately two-fold in the adult CD population (9.09%) compared to the controls (4.08%), it was only 2.63% in the pediatric CD patients (Table 2; $P < 0.05$ comparing the pediatric susceptibility and/or normal variants *versus*

Table 1 Primer sequences and annealing temperatures for genotypings

	SNP	Primers	Tannealing (℃)
NOD2/ CARD15	R702W	F: GAGCCGCACAACCTTCAGATC	50
		R: ACTTGAGGTGCCCAACATTCAG	
	G908R	F: GTTCATGTCTAGAACACATATCAGG	50
		R: GTTCAAAGACCTTCAGAACTGG	
	1007finsC	F: CCTTGAAGCTCACCATTGTATC	50
		R: GATCCTCAAAATTCTGCCATTC	
OCTN1	C1672T	F: AGAGAGTCCTCCTATCTGATTG	54
		R: TCCTAGCTATTCTTCCATGC	
OCTN2	G-207C	F: AGTCCCGCTGCCTTCCTAAG	58
		R: GTCACCTCGTCGTAGTCCCG	

Table 2 Comparison of the alleles of OCTN cation transporters and NOD2/CARD15 genes in pediatric and adult Crohn's disease patients with controls

		Pediatric patients *n* = 19 (%)	Adult patients *n* = 55 (%)	Controls children *n* = 49 (%)
CARD15 genotype				
R702W	CC	18 (94.7)	46 (83.6)	46 (93.9)
	CT	1 (5.3)	8 (14.6)	2 (4.1)
	TT	-	1 (1.8)	1 (2.0)
	T allele frequency (%)	2.63	9.09	4.08
G908R	GG	14 (73.7)	53 (96.4)	48 (98.0)
	GC	3 (15.8)	2 (3.6)	1 (2.0)
	CC	2 (10.5)	-	-
	C allele frequency (%)	18.4	1.82	1.02
1007finsC	- -	13 (68.5)	44 (80.0)	46 (93.9)
	- insC	4 (21.0)	9 (16.4)	3 (6.1)
	insC insC	2 (10.5)	2 (3.6)	-
	Cins allele frequency (%)	21.1%	11.8%	3.06%
SLC22A4 genotype				
C1672T	CC	4 (21.0)	18 (32.7)	12 (24.5)
	CT	11 (58.0)	30 (54.5)	25 (51.0)
	TT	4 (21.0)	7 (12.8)	12 (24.5)
	T allele frequency (%)	50.0	40.0	50.0
SLC22A5 genotype				
G-207C	GG	3 (15.8)	14 (25.5)	10 (20.4)
	GC	7 (36.8)	31 (56.4)	26 (53.1)
	CC	9 (47.4)	10 (18.1)	13 (26.5)
	C allele frequency (%)	65.8	46.4	53.1

the same values of the adult patients or the controls). By contrast, the C allele frequency, encoding the G908R variant, was found highly elevated (18.4%) in pediatric patients, and was only 1.82% in adult CD patients, and 1.02% in the controls ($P < 0.05$). For the 1007finsC variant, a significantly increased prevalence was found both in pediatric (21.1%) and in adult CD (11.8%) patients as compared with the controls (3.06%) ($P < 0.05$).

There were no significant differences in the allele frequencies of SLC22A4 C1672T and SLC22A5 G-207C mutations when compared either the results of the pediatric or the adult CD populations to the results of the controls (Table 2).

DISCUSSION

The carriage rate for the three common CD-associated CARD15 mutations was reported 31% in a pediatric CD population in North America[17], 60% in Germany[18], 51.5% in the Israeli Jewish patients[19] and 40.7% in an Italian cohort[20]. In two studies, the cytosine insertion mutation 3020insC was significantly more common in the pediatric CD population[18,21], whereas among the Jewish patients G908R missense mutation was the most frequent variant[19,22].

An association of the three CARD15 mutations R702W, G908R and 1007finsC with CD has been confirmed in several studies[17,18,23], although different allele frequencies have been observed. While the allele frequencies of the three mutations were almost the same (8.3%, 8.3% and 7.4%) in the Italian pediatric patients[20], the G908R variant was the most frequent among Jewish children[19] and 1007finsC was more common in Germany[18] and in the USA[21]. An earlier onset of disease was found in the presence of a CARD15 mutation in three additional studies[23-25]. These findings suggest that CARD15 mutations may be more frequent in pediatric CD.

To our surprise, in our study groups, two mutations, G908R and 1007finsC, were significantly more frequent in the pediatric population with the allele frequencies of 18.42% in children *versus* 1.02% in controls and of 21.05% in children *versus* 3.06% in controls, respectively. The genotyping results for the adult population are in agreement with previous Hungarian findings[26,27].

The OCTN1 and OCTN2 transporters mediate the transport of carnitine and a wide range of organic cations[28-30] and have an important role in the energy supply of epithelial cells. Recently, it has become clear that the OCTN1 also transports the ergothioneine[31], and the affinity parameters make it almost unquestionable that the carnitine transport function is secondary. A C1672T missense substitution in exon 9 of the SLC22A4 gene results in marked changes in OCTN1 transporter activity, whereas G-207C transversion in the SLC22A5 promoter region causes OCTN2 promoter function impairment.

By resequencing the five genes in the IBD5 interval, which harbors the cytokine gene cluster, and, therefore, is an attractive candidate region for IBD, Peltekova *et al*[16] identified 2 novel polymorphisms in the SLC22A4 and SLC22A5 genes. These two mutations (SLC22A4 C1672T and SLC22A5 G-207C) form a two-allele risk haplotype (OCTN-TC) which was associated with CD and showed significant interactions with CD-associated CARD15 mutations. This observation has been repeatedly confirmed[32-34], although, in the absence of the IBD5 risk haplotype, no association of OCTN1/2 variants with CD was reported in two studies[33,34]. While a Belgian group[35] found that the OCTN did not play a role in the susceptibility to CD, the two functional variants in the SLC22A4 and SLC22A5 genes were completely absent in Japanese[36].

In our study, which is probably the first in the international literature for pediatric population, we could not find accumulation of any of the susceptibility haplotypes either in the pediatric or in the adult CD subjects, thereby not supporting the susceptibility role of the above haplotypes in the development of CD.

In conclusion, we observed an accumulation of CARD15 mutations in pediatric cases, whereas the results for SLC22A4 and SLC22A5 mutation screening do not confirm the assumption that the carriage of these genotypes means significant susceptibility to CD. However, for genotype-phenotype correlations, further studies are needed with larger study populations.

ACKNOWLEDGMENTS

We thank Judit Oksai and Edit Papp for their help in the technical management of the study.

REFERENCES

1 **Oliva-Hemker M**, Fiocchi C. Etiopathogenesis of inflammatory bowel disease: the importance of the pediatric perspective. *Inflamm Bowel Dis* 2002; **8**: 112-128
2 **Baldassano RN**, Piccoli DA. Inflammatory bowel disease in pediatric and adolescent patients. *Gastroenterol Clin North Am* 1999; **28**: 445-458
3 **Gent AE**, Hellier MD, Grace RH, Swarbrick ET, Coggon D. Inflammatory bowel disease and domestic hygiene in infancy. *Lancet* 1994; **343**: 766-767
4 **Hampe J**, Heymann K, Krawczak M, Schreiber S. Association of inflammatory bowel disease with indicators for childhood antigen and infection exposure. *Int J Colorectal Dis* 2003; **18**: 413-417
5 **Hugot JP**, Laurent-Puig P, Gower-Rousseau C, Olson JM, Lee JC, Beaugerie L, Naom I, Dupas JL, Van Gossum A, Orholm M, Bonaiti-Pellie C, Weissenbach J, Mathew CG, Lennard-Jones JE, Cortot A, Colombel JF, Thomas G. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature* 1996; **379**: 821-823
6 **Satsangi J**, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP. Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet* 1996; **14**: 199-202
7 **Cho JH**, Nicolae DL, Gold LH, Fields CT, LaBuda MC, Rohal PM, Pickles MR, Qin L, Fu Y, Mann JS, Kirschner BS, Jabs EW, Weber J, Hanauer SB, Bayless TM, Brant SR. Identification of novel susceptibility loci for inflammatory bowel disease on chromosomes 1p, 3q, and 4q: evidence for epistasis between 1p and IBD1. *Proc Natl Acad Sci USA* 1998; **95**: 7502-7507
8 **Duerr RH**, Barmada MM, Zhang L, Pfützer R, Weeks DE. High-density genome scan in Crohn disease shows confirmed linkage to chromosome 14q11-12. *Am J Hum Genet* 2000; **66**: 1857-1862
9 **Hampe J**, Schreiber S, Shaw SH, Lau KF, Bridger S, Macpherson AJ, Cardon LR, Sakul H, Harris TJ, Buckler A, Hall J, Stokkers P, van Deventer SJ, Nürnberg P, Mirza

MM, Lee JC, Lennard-Jones JE, Mathew CG, Curran ME. A genomewide analysis provides evidence for novel linkages in inflammatory bowel disease in a large European cohort. *Am J Hum Genet* 1999; **64**: 808-816

10 **Rioux JD**, Silverberg MS, Daly MJ, Steinhart AH, McLeod RS, Griffiths AM, Green T, Brettin TS, Stone V, Bull SB, Bitton A, Williams CN, Greenberg GR, Cohen Z, Lander ES, Hudson TJ, Siminovitch KA. Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. *Am J Hum Genet* 2000; **66**: 1863-1870

11 **Rioux JD**, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, Delmonte T, Kocher K, Miller K, Guschwan S, Kulbokas EJ, O'Leary S, Winchester E, Dewar K, Green T, Stone V, Chow C, Cohen A, Langelier D, Lapointe G, Gaudet D, Faith J, Branco N, Bull SB, McLeod RS, Griffiths AM, Bitton A, Greenberg GR, Lander ES, Siminovitch KA, Hudson TJ. Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. *Nat Genet* 2001; **29**: 223-228

12 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599-603

13 **Ogura Y**, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nuñez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606

14 **Hampe J**, Cuthbert A, Croucher PJ, Mirza MM, Mascheretti S, Fisher S, Frenzel H, King K, Hasselmeyer A, MacPherson AJ, Bridger S, van Deventer S, Forbes A, Nikolaus S, Lennard-Jones JE, Foelsch UR, Krawczak M, Lewis C, Schreiber S, Mathew CG. Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. *Lancet* 2001; **357**: 1925-1928

15 **Schreiber S**, Nikolaus S, Hampe J. Activation of nuclear factor kappa B inflammatory bowel disease. *Gut* 1998; **42**: 477-484

16 **Peltekova VD**, Wintle RF, Rubin LA, Amos CI, Huang Q, Gu X, Newman B, Van Oene M, Cescon D, Greenberg G, Griffiths AM, St George-Hyslop PH, Siminovitch KA. Functional variants of OCTN cation transporter genes are associated with Crohn disease. *Nat Genet* 2004; **36**: 471-475

17 **Tomer G**, Ceballos C, Concepcion E, Benkov KJ. NOD2/CARD15 variants are associated with lower weight at diagnosis in children with Crohn's disease. *Am J Gastroenterol* 2003; **98**: 2479-2484

18 **Sun L**, Roesler J, Rösen-Wolff A, Winkler U, Koch R, Thürigen A, Henker J. CARD15 genotype and phenotype analysis in 55 pediatric patients with Crohn disease from Saxony, Germany. *J Pediatr Gastroenterol Nutr* 2003; **37**: 492-497

19 **Weiss B**, Shamir R, Bujanover Y, Waterman M, Hartman C, Fradkin A, Berkowitz D, Weintraub I, Eliakim R, Karban A. NOD2/CARD15 mutation analysis and genotype-phenotype correlation in Jewish pediatric patients compared with adults with Crohn's disease. *J Pediatr* 2004; **145**: 208-212

20 **Ferraris A**, Knafelz D, Torres B, Fortina P, Castro M, Dallapiccola B. Analysis of CARD15 gene variants in Italian pediatric patients with inflammatory bowel diseases. *J Pediatr* 2005; **147**: 272-273

21 **Kugathasan S**, Collins N, Maresso K, Hoffmann RG, Stephens M, Werlin SL, Rudolph C, Broeckel U. CARD15 gene mutations and risk for early surgery in pediatric-onset Crohn's disease. *Clin Gastroenterol Hepatol* 2004; **2**: 1003-1009

22 **Wine E**, Reif SS, Leshinsky-Silver E, Weiss B, Shaoul RR, Shamir R, Wasserman D, Lerner A, Boaz M, Levine A. Pediatric Crohn's disease and growth retardation: the role of genotype, phenotype, and disease severity. *Pediatrics* 2004; **114**: 1281-1286

23 **Lesage S**, Zouali H, Cézard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Binder V, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Merlin F, Chamaillard M, Jannot AS, Thomas G, Hugot JP. CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 2002; **70**: 845-857

24 **Brant SR**, Picco MF, Achkar JP, Bayless TM, Kane SV, Brzezinski A, Nouvet FJ, Bonen D, Karban A, Dassopoulos T, Karaliukas R, Beaty TH, Hanauer SB, Duerr RH, Cho JH. Defining complex contributions of NOD2/CARD15 gene mutations, age at onset, and tobacco use on Crohn's disease phenotypes. *Inflamm Bowel Dis* 2003; **9**: 281-289

25 **Ahmad T**, Armuzzi A, Bunce M, Mulcahy-Hawes K, Marshall SE, Orchard TR, Crawshaw J, Large O, de Silva A, Cook JT, Barnardo M, Cullen S, Welsh KI, Jewell DP. The molecular classification of the clinical manifestations of Crohn's disease. *Gastroenterology* 2002; **122**: 854-866

26 **Büning C**, Molnar T, Nagy F, Lonovics J, Weltrich R, Bochow B, Genschel J, Schmidt H, Lochs H. NOD2/CARD15 gene polymorphism in patients with inflammatory bowel disease: is Hungary different? *World J Gastroenterol* 2005; **11**: 407-411

27 **Lakatos PL**, Lakatos L, Szalay F, Willheim-Polli C, Osterreicher C, Tulassay Z, Molnar T, Reinisch W, Papp J, Mozsik G, Ferenci P. Toll-like receptor 4 and NOD2/CARD15 mutations in Hungarian patients with Crohn's disease: phenotype-genotype correlations. *World J Gastroenterol* 2005; **11**: 1489-1495

28 **Wu X**, George RL, Huang W, Wang H, Conway SJ, Leibach FH, Ganapathy V. Structural and functional characteristics and tissue distribution pattern of rat OCTN1, an organic cation transporter, cloned from placenta. *Biochim Biophys Acta* 2000; **1466**: 315-327

29 **Wu X**, Prasad PD, Leibach FH, Ganapathy V. cDNA sequence, transport function, and genomic organization of human OCTN2, a new member of the organic cation transporter family. *Biochem Biophys Res Commun* 1998; **246**: 589-595

30 **Tamai I**, Ohashi R, Nezu J, Yabuuchi H, Oku A, Shimane M, Sai Y, Tsuji A. Molecular and functional identification of sodium ion-dependent, high affinity human carnitine transporter OCTN2. *J Biol Chem* 1998; **273**: 20378-20382

31 **Gründemann D**, Harlfinger S, Golz S, Geerts A, Lazar A, Berkels R, Jung N, Rubbert A, Schömig E. Discovery of the ergothioneine transporter. *Proc Natl Acad Sci U S A* 2005; **102**: 5256-5261

32 **Newman B**, Gu X, Wintle R, Cescon D, Yazdanpanah M, Liu X, Peltekova V, Van Oene M, Amos CI, Siminovitch KA. A risk haplotype in the Solute Carrier Family 22A4/22A5 gene cluster influences phenotypic expression of Crohn's disease. *Gastroenterology* 2005; **128**: 260-269

33 **Török HP,** Glas J, Tonenchi L, Lohse P, Müller-Myhsok B, Limbersky O, Neugebauer C, Schnitzler F, Seiderer J, Tillack C, Brand S, Brünnler G, Jagiello P, Epplen JT, Griga T, Klein W, Schiemann U, Folwaczny M, Ochsenkühn T, Folwaczny C. Polymorphisms in the DLG5 and OCTN cation transporter genes in Crohn's disease. *Gut* 2005; **54**: 1421-1427

34 **Noble CL**, Nimmo ER, Drummond H, Ho GT, Tenesa A, Smith L, Anderson N, Arnott ID, Satsangi J. The contribution of OCTN1/2 variants within the IBD5 locus to disease susceptibility and severity in Crohn's disease. *Gastroenterology* 2005; **129**: 1854-1864

35 **Vermeire S**, Pierik M, Hlavaty T, Claessens G, van Schuerbeeck N, Joossens S, Ferrante M, Henckaerts L, Bueno de Mesquita M, Vlietinck R, Rutgeerts P. Association of organic cation transporter risk haplotype with perianal penetrating Crohn's disease but not with susceptibility to IBD. *Gastroenterology* 2005; **129**: 1845-1853

36 **Yamazaki K**, Takazoe M, Tanaka T, Ichimori T, Saito S, Iida A, Onouchi Y, Hata A, Nakamura Y. Association analysis of SLC22A4, SLC22A5 and DLG5 in Japanese patients with Crohn disease. *J Hum Genet* 2004; **49**: 664-668

S- Editor Liu Y **L- Editor** Kumar M **E- Editor** Bai SH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5554-5556
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Efficacy of low dose peginterferon alpha-2b with ribavirin on chronic hepatitis C

Rajesh Gupta, CH Ramakrishna, Sandeep Lakhtakia, Manu Tandan, Rupa Banerjee, D Nageshwar Reddy

Rajesh Gupta, CH Ramakrishna, Sandeep Lakhtakia, Manu Tandan, Rupa Banerjee, D Nageshwar Reddy, Department of Medical Gastroenterology and Hepatology, Asian Institute of Gastroenterology, Somajiguda 63661, Hyderabad, India
Correspondence to: D Nageshwar Reddy, Chief Gastroenterologist, Asian Institute of Gastroenterology, Somajiguda 63661, Hyderabad, India. aigindia@yahoo.co.in
Telephone: +91-40-23378888 Fax: +91-40-23324255
Received: 2005-02-22 Accepted: 2005-07-01

Abstract

AIM: To assess the efficacy of peginterferon alpha 2b at doses of 50 μg weekly and 80 μg weekly (based on body weight) plus ribavirin in HCV genotype 2 and genotype 3 chronic hepatitis C patients.

METHODS: During the study period of Jan 2002 to Dec 2003, all patients diagnosed as chronic hepatitis C or HCV related compensated cirrhosis were treated with peginterferon alpha 2b 50 μg S/C weekly (body weight < 60 kg) or 80 μg S/C weekly (body weight > 60 kg) plus ribavirin 800 mg/d for 24 wk.

RESULTS: Overall 28 patients, 14 patients in each group (based on body weight) were treated during the period. Out of 28 patients, 75% were genotype 3, 18% were genotype 2 and 7% were genotype 1. The mean dose of peginterferon alpha 2b was 0.91 μg/kg in group 1 and 1.23 μg/kg in group 2 respectively. The end of treatment and sustained virologic response rates were 82% and 78% respectively. Serious adverse effects were seen in 3.5% patients.

CONCLUSION: Low dose peginterferon alpha 2b in combination with ribavirin for 24 wk is effective in HCV genotype 2 and 3 chronic hepatitis C patients.

Key words: Chronic hepatitis C; Peginterferon alpha 2b; Ribavirin

Gupta R, Ramakrishna CH, Lakhtakia S, Tandan M, Banerjee R, Reddy DN. Efficacy of low dose peginterferon alpha-2b with ribavirin on chronic hepatitis C. *World J Gastroenterol* 2006; 12(34): 5554-5556

http://www.wjgnet.com/1007-9327/12/5554.asp

INTRODUCTION

The hepatitis C virus is a major cause of liver diseases affecting 170 million people worldwide[1]. In India, the estimated prevalence of hepatitis C virus infection is 1.8%. Genotypes 2 and 3 are predominant in Indian population[2].

Therapy for chronic hepatitis C has greatly improved over the last decade and is still evolving[3-8]. The introduction of pegylated form of interferons and addition of ribavirin have further improved the efficacy of therapy in chronic hepatitis C. Higher sustained virological response (SVR) rates in patients with chronic hepatitis C have been reported with pegylated form of interferons compared with standard interferons both as monotherapy as well as in combination with ribavirin[9-13].

Peginterferon alpha-2b is recommended at a dose of 1.5 μg/kg body weight in combination with ribavirin for treatment of chronic hepatitis C. An earlier study has reported similar efficacy of peginterferon alpha-2b at doses of 1 μg/kg and 1.5 μg/kg body weight in treatment of chronic hepatitis C as monotherapy[9]. Recent studies have shown that 82%-90% SVR could be achieved in HCV genotype 2 and 3 patients using peginterferon and ribavirin combination[13,14].

Interferon-based regimens are associated with significant side effects and costs. The cost of therapy in developing countries is a major limiting factor in initiating treatment. There is limited data on efficacy of low dose peginterferon alpha 2b plus ribavirin on chronic hepatitis C. It would be worthwhile to study whether the dose of peginterferon alpha-2b could be lowered from 1.5 μg/kg body weight to 1 μg/kg body weight in combination with standard dose of ribavirin without compromising the efficacy of therapy in HCV genotype 2 and 3 chronic hepatitis C patients.

Therefore, we conducted a non-randomized pilot study to assess the efficacy of peginterferon alpha-2b, at doses of 50 μg and 80 μg (based on body weight) plus ribavirin at standard dose in HCV genotype 2 and 3 related chronic hepatitis and compensated cirrhotic patients.

MATERIALS AND METHODS

Subjects

All patients with chronic liver disease attending our hospital from Jan 2002 to Dec 2003 were evaluated for HCV infection. After detailed history and clinical examination, patients underwent routine hematological investigations, liver function test, abdominal ultrasound

and upper GI endoscopy. All patients were tested for the presence of anti-HCV antibodies with Abbott diagnostic test kit according to the manufacturer's instructions. Those patients who tested positive for anti-HCV were further investigated for HCV RNA. HCV RNA was tested by RT-PCR (SDS) using 30 base pair long dual labeled oligonucleotide TaqMan probe (Rotorgene from Corbett research Australia). HCV genotype was tested by molecular based linear array system using COBAS AMPLICOR.

All patients diagnosed with chronic hepatitis C and compensated cirrhosis were eligible for study. Patients meeting with the following criteria were excluded: Presence of decompensated cirrhosis, hepatitis B coinfection, HIV co-infection, renal failure, concomitant malignancy, co-morbid serious cardiac and respiratory diseases, neuropsychiatric disorders, pregnancy, lactating mothers and alcohol abuse.

Table 1 Demographic, clinical, biochemical and molecular profiles of patients (mean ± SD, *n* = 28)

Demographic profile	
M/F	25:3
Age (yr)	47.5 ± 13.2
Body weight (kg)	60.6 ± 9.18
Clinical profile	
Chronic hepatitis C (*n*)	15
Compensated cirrhosis (*n*)	13
ALT (> 40 IU/L)	18
ALT (< 40 IU/L)	10
Molecular profile	
Genotype 3 (*n*)	21
Genotype 2 (*n*)	5
Genotype 1 (*n*)	2
Follow-up (mo)	18.1 ± 6.7

Study protocol

All eligible patients who gave informed consent were included in the study. Patients were treated with either 50 μg or 80 μg subcutaneous (s/c) weekly dose of pegylated interferon alpha 2b based on body weight plus 800 mg daily dose of ribavirin. Those patients who weighed less than 60 kg were administered a dose of 50 μg per week of pegylated interferon alpha 2b while those weighing more than 60 kg received 80 μg S/C per week. All patients were evaluated as outpatients weekly for 4 wk, then at wk 8, 12, 16 and 24 during treatment. Following the completion of treatment, patients were evaluated at wk 4, 12 and 24. On each visit during follow-up, routine hematological workup was done. Besides, relevant investigations were performed as and when necessary. Qualitative HCV RNA was tested after 12 wk of treatment, at end of treatment (24 wk) and 6 mo after completion of treatment. Liver biopsy was not considered mandatory in study protocol and was done in those patients who agreed to undergo the procedure. Side effects of the therapy were carefully recorded during follow-up. Sustained virologic response was defined as normalization of ALT and negative HCV-RNA 6 mo after completion of therapy.

Informed, written consent was taken from all the patients. Our hospital ethics committee approved the study protocol.

Statistical analysis

The quantitative values were expressed as mean ± SD. Fisher's exact test was used for statistical comparison of the data. $P < 0.05$ was considered as significant.

RESULTS

Overall 28 patients (25 males and 3 females) were included in the study. The demographic profile and clinical characteristics of the patients are shown in Table 1.

Fifteen out of 28 patients (54%) were chronic hepatitis and the remaining 13 (46%) were compensated cirrhosis (Child A). Eighteen (64%) of 28 patients had elevated ALT > 1.5 times the upper limit. The pretreatment ALT levels were 89 IU/L ± 28 IU/L. Out of 28 patients, 75%, 18% and 7% were genotype 3, 2 and 1 respectively. Fourteen patients (mass < 60 kg) were administered 50 μg S/C weekly dose of peginterferon alpha 2b plus 80 mg oral daily dose of ribavirin. The mean dose of peginterferon alpha 2b was 0.91 μg/ kg body weight (0.6 μg/kg ± 1.22 μg/kg) in this group.

The second group of 14 patients (mass > 60 kg) received 80 μg S/C weekly dose of peginterferon alpha 2b with daily oral dose of 800 mg ribavirin. The mean dose of peginterferon alpha 2b in this group was 1.23 μg/kg (1.03 μg/kg ± 1.33 μg/kg). One patient discontinued treatment due to severe side effects and another patient lost to follow up after 2 wk. Twenty-six out of 28 patients completed the 24 wk treatment. At the end of 24 wk treatment, 23 patients were negative for HCV RNA and 3 patients tested positive for HCV RNA. On further follow up, one patient tested positive for HCV RNA 6 mo after completion of therapy. Overall, at the end of treatment, 23 (82%) of 28 patients showed response to therapy. The SVR was achieved in 22 (78%) out of 28 patients. Though the dose per kg body weight of peginterferon alpha 2b was significantly different in two groups ($P < 0.002$), there was no difference in response to therapy with respect to dose or genotype (Table 2). During the follow-up of 18.1 ± 6.7 mo, one non-responder patient developed hepatocarcinoma (HCC) and died of liver failure following variceal bleeding.

One patient (3.5%) developed unbearable weakness and burning in urethra and discontinued the treatment. Seven (25%) of 28 patients developed mild leucopenia and thrombocytopenia which required temporary interruption of treatment for 1-2 wk. However, none of these 7 patients discontinued treatment any more and completed the 24 wk treatment.

DISCUSSION

The combination of peginterferon and ribavirin is the mainstay of treatment in chronic hepatitis C. Both forms of peginterferon alpha 2a and alpha 2b in combination with ribavirin have shown SVR rates from 79%-93% in genotype 2 and 3 chronic hepatitis C patients, making it a potentially curable disease[14,15]. In our study, end of treatment response (ETR) and SVR rates were 82%

Table 2 Response of therapy with respect to dose and genotype

Patients (n = 28)	Response to peginterferon alpha 2b Dose 50 mcg (n = 14)		Response to peginterferon alpha 2b Dose 80 mcg (n = 12)[1]	
	Yes	No	Yes	No
Genotype 1 (n = 2)	-	1	1	-
Genotype 2 (n = 5)	3	-	2	-
Genotype 3 (n = 21)	8	2	8	1

[1]Two patients in group 2 did not complete therapy.

and 78% respectively. The mean dose per kg body weight of peginterferon alpha 2b was 0.9 μg/kg and 1.23 μg/kg in the two groups, respectively. Lindsay *et al*[9] have shown similar SVR rates with 1.5 μg/kg and 1 μg/kg of peginterferon alpha 2b as monotherapy.

In a recent study, Zeuzem *et al*[14] have shown SVR rates of 79% and 93% in genotype 3 and genotype 2 chronic hepatitis C patients respectively when treated with peginterferon alpha 2b 1.5 μg/kg S/C weekly plus ribavirin 800-1400 mg/d based on body weight. In the same study, higher virologic response rate was observed in HCV genotype 3 patients with baseline HCV RNA concentration of < 600 IU/L compared to those with baseline HCV RNA > 600 IU/L (85% *vs* 59%). In our study, quantitative HCV RNA assay was not included in the study protocol, hence data could not be analysed based on HCV RNA levels. However, our results are comparable with those reported by Zeuzem *et al*[14], albeit at a much lower dose. One patient (3.5%) reported serious side effects resulting in discontinuation of treatment. The remaining patients tolerated the treatment well. The overall safety profile was much improved compared with earlier studies[16]. One patient who was a non-responder to treatment died during follow-up due to HCC related decompensation, suggestive of progressive disease.

HCV genotypes 1b and 2a are common in China[15]. In Japan, genotypes 1b, 2a and 2b are prevalent. Genotype 3 is also observed in Southeast Asian countries. In India, genotypes 2 and 3 are predominant HCV genotypes[2]. These genotypes 2 and 3 respond well to treatment[11,13,14]. But the cost of the treatment is a major limiting factor in developing countries. Our study has shown that > 80% SVR rates could be achieved in HCV genotype 2 and 3 infected chronic hepatitis C patients with lower than recommended dose of pegylated interferon alpha 2b. In light of encouraging results of our study, a randomized controlled trial is needed to compare the efficacy of lower doses of pegylated interferon alpha 2b, ie, with 1.5 μg/kg body weight of peginterferon alpha 2b in combination with standard dose of ribavirin with specific reference to Asian population.

In conclusion, the present study shows that lower than recommended dose of peginterferon alpha 2b in combination with ribavirin for 24 wk is effective in HCV genotype 2 and 3 chronic hepatitis C patients.

REFERENCES

1 **Global surveillance and control of hepatitis C**. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. *J Viral Hepat* 1999; **6**: 35-47

2 **Das BR**, Kundu B, Khandapkar R, Sahni S. Geographical distribution of hepatitis C virus genotypes in India. *Indian J Pathol Microbiol* 2002; **45**: 323-328

3 **Lauer GM**, Walker BD. Hepatitis C virus infection. *N Engl J Med* 2001; **345**: 41-52

4 **Seeff LB**. Natural history of chronic hepatitis C. *Hepatology* 2002; **36**: S35-S46

5 **Marcellin P**, Boyer N, Gervais A, Martinot M, Pouteau M, Castelnau C, Kilani A, Areias J, Auperin A, Benhamou JP, Degott C, Erlinger S. Long-term histologic improvement and loss of detectable intrahepatic HCV RNA in patients with chronic hepatitis C and sustained response to interferon-alpha therapy. *Ann Intern Med* 1997; **127**: 875-881

6 **Poynard T**, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zeuzem S, Trepo C, Albrecht J. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. *International Hepatitis Interventional Therapy Group (IHIT) Lance*t 1998; **352**: 1426-1432

7 **McHutchison JG**, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, Goodman ZD, Ling MH, Cort S, Albrecht JK. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; **339**: 1485-1492

8 National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C: 2002--June 10-12, 2002. *Hepatology* 2002; **36**: S3-20

9 **Lindsay KL**, Trepo C, Heintges T, Shiffman ML, Gordon SC, Hoefs JC, Schiff ER, Goodman ZD, Laughlin M, Yao R, Albrecht JK. A randomized, double-blind trial comparing pegylated interferon alfa-2b to interferon alfa-2b as initial treatment for chronic hepatitis C. *Hepatology* 2001; **34**: 395-403

10 **Zeuzem S**, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, O'Grady J, Reichen J, Diago M, Lin A, Hoffman J, Brunda MJ. Peginterferon alfa-2a in patients with chronic hepatitis C. *N Engl J Med* 2000; **343**: 1666-1672

11 **Manns MP**, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965

12 **Fried MW**, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales FL, Häussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975-982

13 **Hadziyannis SJ**, Sette H Jr, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H Jr, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346-355

14 **Zeuzem S**, Hultcrantz R, Bourliere M, Goeser T, Marcellin P, Sanchez-Tapias J, Sarrazin C, Harvey J, Brass C, Albrecht J. Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. *J Hepatol* 2004; **40**: 993-999

15 **Fan WM**, Zhu WF, Yin LM, Wei L, Xu XY, Zhuang H. Prospective study in 142 cases of hepatitis C virus infection. *World J Gastroenterol* 2004; **10**: 2867-2869

16 **Fried MW**. Side effects of therapy of hepatitis C and their management. *Hepatology* 2002; **36**: S237-S244

S- Editor Wang GP **L- Editor** Zhu LH **E- Editor** Bi L

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5557-5561
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Enhanced expression of epidermal growth factor receptor gene in gastric mucosal cells by the serum derived from rats treated with electroacupuncture at stomach meridian acupoints

Zong-Bao Yang, Jie Yan, Xiao-Ping Zou, Shou-Xiang Yi, Xiao-Rong Chang, Ya-Ping Lin, Xi-Ping Li

Zong-Bao Yang, Jie Yan, Xiao-Ping Zou, Shou-Xiang Yi, Xiao-Rong Chang, Ya-Ping Lin, Xi-Ping Li, College of Acupuncture and Moxibustion, Hunan University of Traditional Chinese Medicine, Changsha 410007, Hunan Province, China
Supported by the National Natural Science Foundation of China, No. 90209023; the State 973 Project, No. 2005CB523308
Correspondence to: Professor Jie Yan, College of Acupuncture and Moxibustion, Hunan University of Traditional Chinese Medicine, Changsha 410007, Hunan Province, China. yj5381159@yahoo.com.cn
Telephone: +86-731-5381159 Fax: +86-731-5381159
Received: 2006-05-11 Accepted: 2006-06-14

Abstract

AIM: To investigate the effect of serum derived from rats treated with electroacupuncture at stomach meridian acupoints on the expression of epidermal growth factor receptor (EGFR) gene in gastric mucosal cells.

METHODS: The stress-induced gastric mucosal injury in rat model was established by water-immersion and restrained stress methods. 52 rats were randomly divided into: normal group (n = 8), model group (n = 8), model serum group (n = 12), stomach serum group (n = 12), and gallbladder serum group (n = 12). The gastric mucosal cells were separated by pronase-EDTA digestion method and incubated with serum. The EGFR gene expression in gastric mucosal cells was detected by reverse transcription-polymerase chain reaction (RT-PCR) method.

RESULTS: Compared with normal group (0.6860 ± 0.0594), the serum derived from rats of the stomach group (1.2272 ± 0.0813, P = 0.00 < 0.01) and gallbladder group (0.9640 ± 0.0387, P = 0.00 < 0.01) had a tendency to enhance the EGFR gene expression in gastric mucosal cells. Such tendency existed in the model group (0.7104 ± 0.0457) but with no significant difference (P = 0.495 > 0.05) and in model serum group (0.8516 ± 0.0409) with an extremely obvious difference (P = 0.001 < 0.01). Furthermore, the EGFR gene expression in stomach serum group was significantly higher than that in gallbladder serum group (P = 0.00 < 0.01).

CONCLUSION: The present study shows that serum derived from rats treated with electroacupuncture at stomach meridian acupoints can distinctly increase the EGFR gene expression of gastric mucosal cells. Therefore, there is certain meridian specificity in the serum, which could provide a proof for the TCM theory "particular relation between meridian and internal organ".

Key words: Electroacupuncture; Serum; Stomach meridian acupoints; Gastric mucosal cells; Epidermal growth Factor receptor; Gene expression

Yang ZB, Yan J, Zou XP, Yi SX, Chang XR, Lin YP, Li XP. Enhanced expression of epidermal growth factor receptor gene in gastric mucosal cells by the serum derived from rats treated with electroacupuncture at stomach meridian acupoints. *World J Gastroenterol* 2006; 12(34): 5557-5561

http://www.wjgnet.com/1007-9327/12/5557.asp

INTRODUCTION

Gastric mucosal damage is a common pathological reaction in the diseases of the digestive system. The acupuncture and moxibustion are very effective cure for this damage[1,2]. Previous experimental studies demonstrated that epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) were the most important peptides for the repair of the gastric mucosal injury[3]. Acupuncture at gastric meridian acupoint could alter gastric motility and secretion and also the content of gastrin, substance P, EGF and TGF-α in serum and gastric mucosa[4,5]. Recent research indicated that EGFR was closely related to the healing of impaired gastric mucosa, which was of great importance to the gastric mucosal protection and repair after damage[6]. The EGFR belongs to the family of trans-membrane tyrosine protein kinase (TPK). Activation of EGFR stimulates cell proliferation, differentiation, adhesion, and migration[7,8]. The aim of this study was to examine the effect of serum derived from rats treated with electroacupuncture at stomach meridian acupoints on the expression of EGFR gene in gastric mucosal cells. This would hopefully clarify the humoral

mechanism of acupuncture effect on gastric mucosal cells and the essential correlation of the meridian acupoints and internal organs.

MATERIALS AND METHODS

Reagents

Pronase and dithiothreitol (DTT) were purchased from MERK. Bovine serum albumin (BSA) was obtained from Biosharp. Percoll was purchased from Pharmacia, Dulbecco's Modified Eagle Medium (DMEM) from Hyclone, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) from Biosource, Trizol reagent was obtained from Invitrogen. AMV reverse transcriptase, ribonuclease inhibitor (RNasin), dNTPs, Taq DNA polymerase, 100 bp DNA ladder, diethylpyrocarbonate (DEPC), oligodT18 primer, and gelose were purchased from Promega. Tyrosine kinase inhibitor (PD153035) was purchased from Calbiochem. EGFR and the internal control, glyseraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from Invitrogen. All other reagents were analytically pure.

The stress-induced gastric mucosal injury in rats

Water-immersion and restrained stress methods were adopted[9]. Before modelling, the experimental rats were fasted for 24 h and had free access to water only. Rats were fixed on boards and were immersed vertically in a homeostatic bath at 23 ± 1℃ for 10 h, with the liquid surface up to the level of the xiphoid process of the sternum.

Experimental design

Sprague-Dawley (SD) male and female rats, with an average weight of 200 ± 30 gm, were supplied by the Experimental Animal Center at Hunan Agriculture University (Permission number: 20030316) 2 wk before the experiment. During this period, they had access to Purina rat chow and water. Animals were fasted overnight before the experiments. Fifty-two rats were randomly divided into normal group, model group, model serum group, stomach serum group and gallbladder serum group. 8 rats were in the normal group and model group. Each of the model serum group, stomach serum group and gallbladder serum group included 12 rats.

Four rats of each of the model serum group, stomach serum group, and gallbladder serum group were selected at random for deriving serum, and the remaining 8 rats were used for isolating gastric mucosal cells. Acupoints location was defined by reference of rat-acupoint-atlas and analogy to human body[10]. According to the induction stated above, three pairs of acupoints consisting of Sibai (ST 2), Liangmen (ST 21), and Zusanli (ST36) in the stomach Meridian, were designed, which represent acupoints of different level (head, trunk, and limb). Also, 3 pairs of acupoints of the gallbladder Meridian in the same horizontal level were selected: Yangbai (GB 14), Riyue (GB24), and Yanglingquan (GB 34).

Pairs of stainless-steel needles of 0.25 mm in diameter were inserted into the acupoints stated above in experimental rats. The needles were connected to the output of an electronic pulse generator, a medical electroacupuncture stimulator (Model G6805-1, made by Shanghai Medical Electro-apparatus Factory, China), which achieves intermittent-and-irregular wave (intermittent wave: 4 Hz, irregular wave: 20 Hz), constant time of 30 min per day, ten days, while there was a light vibration in the lower limbs of rats.

Isolation of gastric mucosal cells

Animals were fasted overnight before the experiments. All experiments were performed using freshly isolated gastric mucosal cells. The contents of the stomach were washed out with phosphate-buffered saline (PBS). The stomach was then ligated at the base of the forestomach and the proximal end of the antrum to obtain mucosal cells primarily from the oxyntic region. After being transformed into inside-out gastric bags, they were filled with 2.5 mL of 1 mg/mL pronase solution in buffer A (0.5 mmol/L NaH_2PO_4, 1.0 mmol/L Na_2HPO_4, 20 mmol/L $NaHCO_3$, 80 mmol/L NaCl, 5.0 mmol/L KCl, 50 mmol/L HEPES, 11 mmol/L glucose, 0.02 mmol/L BSA, 2 mmol/L EDTA, pH 7.4). The filled gastric bags were incubated in pronase-free buffer A at 37℃ for 30 min. The gastric bags were then transferred into buffer B (0.5 mmol/L NaH_2PO_4, 1.0 mmol/L Na_2HPO_4, 20 mmol/L $NaHCO_3$, 80 mmol/L NaCl, 5.0 mmol/L KCl, 50 mmol/L HEPES, 11 mmol/L glucose, 0.01 mmol/L BSA, 1 mmol/L CaCl, 1.5 mmol/L MgCl, pH7.4) and gently agitated by a magnetic stirrer at room temperature for 1h. The gastric mucosal cells dispersed in buffer B were collected by centrifuging at 3000 rpm for 5 min and subsequently resuspended in serum-free DMEM[11,12].

Serum collection

The blood was sampled from carotid artery after rats were treated according to the requirement of experimental procedures. Then the blood was transferred into centrifuge tubes and placed steadily for 2 h at 37℃. Tubes were centrifuged at 2500 rpm for 10 min. The serum was carefully sucked and frozen at -20℃. The gastric mucosal cells were incubated with 100 mL/L serum at 37℃ for 30 min in the experiment[13,14].

RNA extraction

Following the treatment stated above, gastric mucosal cells obtained from each rat were collected in Eppendorf tubes and kept in the -80℃. Eight samples from each group were selected randomly for RNA extraction. Total RNA was isolated from samples of gastric mucosal cells by using a guanidium isothiocyanate/phenol chloroform single step extraction kit from Stratagene. Total RNA was precipitated in ethanol and resuspended in sterile RNAase-free water for storage at -80℃ untill use. Total RNA was quantified spectrometrically at 260 nm, and the quality of isolated RNA was analyzed on agarose gels under standard conditions.

Reverse transcription reaction

Total RNA (10 μL, about 0.5 μg/sample) was reverse transcribed (RT) using oligo (dT) 18 primers 1 μL, 5 ×

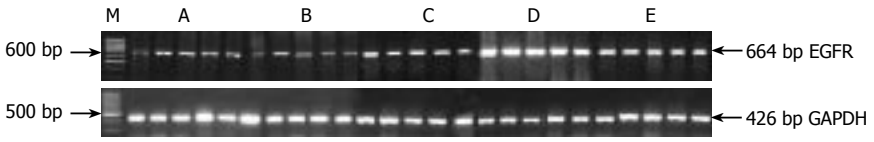


Figure 1 Electrophoresis of EGFR mRNA and GAPDH mRNA RT-PCR product in gastric mucosal cells. M: Marker; A: Normal group; B: Model group; C: Model serum group; D: Stomach serum group; E: Gallbladder serum group.

Table 1 The primer sequences and sizes of amplification products

EGFR	Forward primer: 5'-AGT GGT CCT TGG AAA CTT GG-3'	664 bp
	Reverse primer: 5'- GTT GAC ATC CAT CTG GTA CG-3'	
GADPH	Forward primer: 5'-TGC TGA GTA TGT CGT GGA GTC -3'	426 bp
	Reverse primer: 5'-AAG GCC ATG CCA GTG AGC TTC -3'	

Table 2 The EGFR gene expression in gastric mucosal cells (mean ± SD, n = 8)

Group	EGFR mRNA/GAPDH mRNA
Normal group	0.6860 ± 0.0594
Model group	0.7104 ± 0.0457[d]
Model serum group	0.8516 ± 0.0409[b,d]
Stomach serum group	1.2272 ± 0.0813[b]
Gallbladder serum group	0.9640 ± 0.0387[b,d]

[b]$P < 0.01$ *vs* Model group; [d]$P < 0.01$ *vs* Stomach serum group.

RT-buffer 4 μL, dNTPs (10 mmol/L) 1 μL, RNasin (20 MU/μL) 0.5 μL, M-MULV reverse transcriptase (200 MU/μL) 1 μL, and DEPC-treated water 2.5 μL in a 20 μL reverse transcription reaction system. The reaction was performed at 42℃ for 30-60 min so that target mRNA was transcribed into cDNA. The tubes were cooled and centrifuged for several seconds.

Polymerase chain reaction (PCR)

An aliquot of the RT product of each sample (1/20 of the total volume) was used in the PCR amplification reactions for EGFR and GAPDH. The PCR reaction contained 4 μL cDNA, 10 × PCR buffer 5 μL, dNTPS (10 mmol/L) 1 μL, oligonucleotide primers sense/antisense (10 mmol/L) 1 μL (primer sequences are stated below), Taqase 1 μL, ddH2O 32 μL in a total volume of 50 μL. Reaction mixtures were incubated for predenaturation at 94℃ for 2 min, followed by 38 cycles for EGFR (denaturation at 94℃ for 30 s, annealing at 53℃ for 1 min, and extension at 72℃ for 1 min) and 25 cycles for GAPDH (denaturation at 94℃ for 30 s, annealing at 56℃ for 30 s, and extension at 72℃ for 30 s), and a final extension at 72℃ for 5 min.

Primer design and the RT-PCR product electrophoresis

To use of the relatively quantitative method to measure EGFR gene expression, rat GAPDH was selected as internal control substance. The primer sequences and sizes of amplification products are as shown in Table 1. Five microliter PCR products were analyzed on 10 g/L agarose gel containing ethidiumbromide with TBE buffer at 80 V for 40 min and photographed under UV illumination. The band intensities were quantified by densitometry. EGFR and GAPDH PCR products were, respectively, 664 and 426 base pairs (Table 1). EGFR and GAPDH were determined by computer-assisted densitometric scanning. Signals were quantified by density analysis of the digital images using Eagle Eye Ⅱ image software (Stratagene) and EGFR/GAPDH quotient indicated the relative expression of EGFR. Experiments were performed in triplicate.

Statistical analysis

The data for each group were expressed as mean ± SD. Comparison between groups was assessed using one-way analysis of variance (ANOVA). Differences were considered statistically significant if the P value was less than 0.05. Software SPSS 13.0 was used in all statistical tests.

RESULTS

Expression of EGFR gene in gastric mucosal cells

By using RT-PCR, the EGFR gene expression in gastric mucosal cells were detected in rats of normal group and model group as a weak signal but it was well-defined among other groups: model serum group, stomach serum group and gallbladder serum group. Compared with Model serum group, the serum in stomach serum group and gallbladder serum group appeared to up-regulate significantly the EGFR gene expression in gastric mucosal cells, $P < 0.01$, and obvious difference between stomach serum group and gallbladder serum group was found ($P < 0.01$). However, there was no difference between normal group and model group, $P > 0.05$ (Table 2; Figure 1).

DISCUSSION

According to the classical Traditional Chinese Medicine (TCM) theory, there is a particular relation between meridian acupoints and viscera and the functional activities of the organism can be regulated by acupuncture at the meridian acupoints. However, it is still unknown how the acupuncture regulates the functional activities of the organism, and what is essential for the relationship between meridian acupoints and viscera. The present study proved that the acupuncture at the stomach meridian acupoints could improve gastric mucosal protection mechanism and that it is a very effective cure for gastrointestinal diseases[15,16]. Acupuncture at acupoints of Sibai (ST2), Liangmen (ST21), and Zusanli (ST36), could produce certain ameliorative effect through the following mechanisms: augmentation of the gastric antrum, reinforcement of pressure on gastric pyloric sphincter, stimulation or inhibition of related gastrointestinal peptide secretion[17,18]. All of these have provided experimental

evidence for the theory of "Particular relationship between gastric meridian and the stomach". However, the functional mechanism of the repair of gastric mucosal lesion is not entirely clear, and the humoral factor of acupuncture and moxibustion effect is still unknown.

The mucosal lining of the gastrointestinal tract, especially the stomach, is easily exposed to a variety of exogenous injurious agents, including non-steroidal anti-inflammatory drugs and ethanol. Each of these agents either alone or in combination with others may induce mucosal injury. However, a number of *in vivo* and *in vitro* studies have demonstrated that the gastric mucosa of animals possesses the inherent capacity to repair after mild injury[19]. The cellular protective functions against damage maybe accomplished in several ways. There are evidences for participation of both the early phase of epithelial repair, known as restitution, marked by increased cell migration but no proliferation, and the delayed phase of cell renewal, marked by proliferation, differentiation and migration[20,21].

In general, EGFR is one of the recently described members of cell membrane proteins. It is made of 1186 amino acids. As a trans-membrane receptor of tyrosine protein kinase family, EGFR plays a very important role in regulating healing process of damaged gastric mucosa, and regulates cell metabolism, proliferation, differentiation, migration and other biological phenomena. Many studies indicated that there was an elevated EGFR expression during the healing course of damaged gastric mucosa. Therefore, EGFR is of a great importance to the gastric mucosal protection and injury healing[22,23]. The relationship between EGFR and its downstream signal transduction pathway and the healing of gastric mucosal injury is increasingly becoming a focus of researchers' attention. This study assessed, by RT-PCR methods, the EGFR mRNA expression in gastric mucosal cells of the rat after incubation with 10% serum for 30 min. The data showed that EGFR mRNA expression in gastric mucosal cells was enhanced shortly after incubation with the serum derived from the rats. Meanwhile, it was proved that the serum derived from the rats treated with electroacupuncture had an obvious tendency to stimulate the EGFR mRNA expression in gastric mucosal cells. In addition, EGFR mRNA expression in stomach serum group was much higher than that in model serum group and gallbladder serum group. Therefore, we hypothesize that the serum derived from rats treated with electroacupuncture contains many kinds of active substances that stimulated the EGFR gene in the gastric mucosal cells. This study also indicated that the discrepancy in the expression of EGFR gene may be the underlying mechanism of different effect of electroacupuncture at acupoints of gastric meridian and that of gallbladder meridian. Thus, this could be a proof for the TCM theory "particular relation between SMFY and the stomach". The active substance (s) in the serum derived from the rats treated with electroacupuncture at stomach meridian acupoints is (are) unknown, and therefore, more research using proteomic technology is needed.

REFERENCES

1 **Wan Q**. Auricular-plaster therapy plus acupuncture at zusanli for postoperative recovery of intestinal function. *J Tradit Chin Med* 2000; **20**: 134-135
2 **Butov MA**, Alebastrov AP, Kuznetsov PS, Shirokova IV. [The age aspect of treatment of patient with ulcer of gastroduodenal zone]. *Adv Gerontol* 2004; **14**: 96-100
3 Gönül **B**, Akbulut KG, Ozer C, Yetkin G, Celebi N. The role of transforming growth factor alpha formulation on aspirin-induced ulcer healing and oxidant stress in the gastric mucosa. *Surg Today* 2004; **34**: 1035-1040
4 **Li H**, Tang CZ, Li SH, Zhang Z, Chen SJ, Zhang JW. [Effects of thread embedding therapy on nucleotides and gastrointestinal hormones in the patient of chronic gastritis]. *Zhongguo Zhen Jiu* 2005; **25**: 301-303
5 **Chang CH**, Huang JL, Ting CT, Chang CS, Chen GH. Atropine-induced HRV alteration is not amended by electroacupuncture on Zusanli. *Am J Chin Med* 2005; **33**: 307-314
6 **Tarnawski AS**, Jones MK. The role of epidermal growth factor (EGF) and its receptor in mucosal protection, adaptation to injury, and ulcer healing: involvement of EGF-R signal transduction pathways. *J Clin Gastroenterol* 1998; **27** Suppl 1: S12-S20
7 **Mendelsohn J**. Targeting the epidermal growth factor receptor for cancer therapy. *J Clin Oncol* 2002; **20**: 1S-13S
8 **Ciardiello F**, Tortora G. A novel approach in the treatment of cancer: targeting the epidermal growth factor receptor. *Clin Cancer Res* 2001; **7**: 2958-2970
9 **Konturek PC**, Brzozowski T, Konturek SJ, Taut A, Sliwowski Z, Stachura J, Hahn EG. Activation of genes for growth factors and cyclooxygenases in rat gastric mucosa during recovery from stress damage. *Eur J Pharmacol* 1998; **342**: 55-65
10 **He J**, Yan J, Chang X, Liu J, Li J, Yi S, Lin Y. Neurons in the NTS of rat response to gastric distention stimulation and acupuncture at body surface points. *Am J Chin Med* 2006; **34**: 427-433
11 **Everett SM**, White KL, Schorah CJ, Calvert RJ, Skinner C, Miller D, Axon AT. In vivo DNA damage in gastric epithelial cells. *Mutat Res* 2000; **468**: 73-85
12 **Xiao ZQ,** Majumdar AP. Induction of transcriptional activity of AP-1 and NF-kappaB in the gastric mucosa during aging. *Am J Physiol Gastrointest Liver Physiol* 2000; 278: G855-G865
13 **Chen Y**, Zhao C, Chen H, Qin H, Fang F. Effects of "moxibustion serum" on proliferation and phenotypes of tumor infiltrating lymphocytes. *J Tradit Chin Med* 2003; 23: 225-229
14 **Luo MF**, Li CH, Zhang JL, Guo Y, Chen SP, Liu JL, Li RW. [Acupuncture-serum decreases Ca2+ content in cultured rat myocardial cells]. *Zhongguo Zhen Jiu* 2006; **26**: 367-370
15 **Iwa M**, Nakade Y, Pappas TN, Takahashi T. Electroacupuncture improves restraint stress-induced delay of gastric emptying via central glutaminergic pathways in conscious rats. *Neurosci Lett* 2006; **399**: 6-10
16 **Lee YH**, Lee MS, Shin BC, Jeong JS, Jeong DM, Hwang IC, Kim JI. Effects of acupuncture on potential along meridians of healthy subjects and patients with gastric disease. *Am J Chin Med* 2005; **33**: 879-885
17 **Yan J**, Yang RD, He JF, Yi SX, Chang XR, Lin YP. Effect of acupuncture at different meridian acupoints on changes of related factors for rabbit gastric mucosal injury. *World J Gastroenterol* 2005; **11**: 6472-6476
18 **Li XP**, Yan J, Yi SX, Chang XR, Lin YP, Yang ZB, Huang A, Hu R. Effect of electroacupunture on gastric mucosal intestinal trefoil factor gene expression of stress-induced gastric mucosal injury in rats. *World J Gastroenterol* 2006; **12**: 1962-1965
19 **McNeil PL**. Repairing a torn cell surface: make way, lysosomes to the rescue. *J Cell Sci* 2002; **115**: 873-879
20 **Sobue M**, Joh T, Oshima T, Suzuki H, Seno K, Kasugai K, Nomura T, Ohara H, Yokoyama Y, Itoh M. Contribution of

capsaicin-sensitive afferent nerves to rapid recovery from ethanol-induced gastric epithelial damage in rats. *J Gastroenterol Hepatol* 2003; **18**: 1188-1195

21 **Rodríguez JA,** Theoduloz C, Yáñez T, Becerra J, Schmeda-Hirschmann G. Gastroprotective and ulcer healing effect of ferruginol in mice and rats: assessment of its mechanism of action using in vitro models. *Life Sci* 2006; **78**: 2503-2509

22 **Zhang WN**, Zhang Y, Li JB. [Effect of Jianwei Yuyang Granules on expression of epidermal growth factor receptor in gastric mucosa of gastric ulcer patients]. *Zhongxiyi Jiehe Xuebao* 2004; **2**: 24-26

23 **Fujiwara Y**, Higuchi K, Hamaguchi M, Takashima T, Watanabe T, Tominaga K, Oshitani N, Matsumoto T, Arakawa T. Increased expression of transforming growth factor-alpha and epidermal growth factor receptors in rat chronic reflux esophagitis. *J Gastroenterol Hepatol* 2004; **19**: 521-527

S- Editor Liu Y **L- Editor** Karam SM **E- Editor** Bi L

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5562-5564
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Percutaneous transarterial embolization of extrahepatic arteroportal fistula

Gianluca Marrone, Settimo Caruso, Roberto Miraglia, Ilaria Tarantino, Riccardo Volpes, Angelo Luca

Gianluca Marrone, Settimo Caruso, Roberto Miraglia, Ilaria Tarantino, Riccardo Volpes, Angelo Luca, Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione (IsMeTT), Palermo, Italy
Correspondence to: Roberto Miraglia, Radiology Department, IsMeTT, Via Carmelo Lazzaro 2a, CAP 90127, Palermo, Italy. rmiraglia@ismett.edu
Telephone: +39-91-2192293 Fax: +39-91-2192344
Received: 2006-05-05 Accepted: 2006-06-14

Abstract

Arteroportal fistula is a rare cause of prehepatic portal hypertension. A 44-year-old male with hepatitis virus C infection was admitted for acute variceal bleeding. Endoscopy showed the presence of large esophageal varices. The ultrasound revealed a mass near the head of pancreas, which was characterized at the color-Doppler by a turbulent flow, and arterialization of portal vein flow. CT scan of abdomen showed a large aneurysm of the gastroduodenal artery communicating into the superior mesenteric vein. The sinusoidal portal pressure measured as hepatic vein pressure gradient was normal, confirming the pre-hepatic origin of portal hypertension. The diagnosis of extrahepatic portal hypertension secondary to arteroportal fistula was established, and the percutaneous embolization was performed. Three months later, the endoscopy showed absence of esophageal varices and ascites. At the moment, the patient is in good clinical condition, without signs of portal hypertension.

Key words: Portal hypertension; Arteroportal fistula; Portal shunt; Esophageal varices bleeding; Embolization; Interventional radiology; Psudoaneurysm

Marrone G, Caruso S, Miraglia R, Tarantino I, Volpes R, Luca A. Percutaneous transarterial embolization of extrahepatic arteroportal fistula. *World J Gastroenterol* 2006; 12(34): 5562-5564

http://www.wjgnet.com/1007-9327/12/5562.asp

INTRODUCTION

Extrahepatic arteroportal fistula is a rare disorder of hepatic vasculature characterized by anomalous communication between arteries and portal vein system most commonly caused by abdominal trauma, and iatrogenic damage induced by procedures such as liver biopsies, tumors and congenital vascular malformations[1]. Arteroportal fistula may cause severe portal hypertension which leads to gastroesophageal variceal bleeding, refractory ascites, diarrhea, and hepatic encephalopathy. The recent progress in computed tomography and magnetic resonance technology with no invasive angiography imaging , makes it possible to confirm the suspicious arteroportal fistula at the Doppler ultrasonography and guide the choice of the treatment. The hepatic vein portal pressure gradient (HVPG) measurement may verify the diagnosis of extrahepatic portal hypertension.

CASE REPORT

A 44-year-old male was admitted to our Institution because of acute variceal bleeding and a clinical diagnosis of cirrhosis. The patient underwent a total gastrectomy in 1984 for gastroduodenal ulcer. In December 2000, there was a biopsy-proven chronic active hepatitis secondary to hepatitis virus C infection; and a long-term biochemical and virological response was obtained with a 12-mo course of combined antiviral treatment with PEG-IFN plus Ribavirine. Upon admission, the liver function tests were made and the results were: AST 34 U/L, ALT 61 U/L, INR 1.2, PT 63%, serum albumin 33 g/L, serum bilirubin 1.1 mg/dL, and platelets count 15×10^3 μL.

The endoscopy showed the presence of large esophageal varices with cherry red spots, localized in the medial and lower third of the esophagus and extended into the subcardial tract; the varix actively bleeding was treated with banding legations. Two days later, the ultrasonography showed homogeneous liver texture, enlarged spleen (a longitudinal diameter of 16.3 cm) and absence of ascites. However, an anechoic mass 2.5 cm in diameter was detected next to the head of the pancreas. The color-Doppler demonstrated turbulent flow, and arterialization of portal vein flow, with an increased peak velocity (83 cm/sec) (Figure 1). All these findings were suggestive for extrahepatic arteroportal fistula. A 16 slices-MDCT scan of the abdomen was performed, using 150 mL of isosmolate iodate contrast media (1.8 mL/kg), injection-velocity of 5 mL/sec, pitch 1, thickness of 2.5 mm, interval reconstruction of 0.6 mm, and triple-phase acquisition. Maximum Intensity Projection

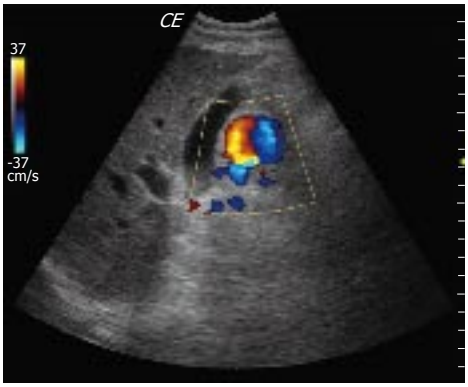


Figure 1 Color –Doppler US showed an anechoic mass, 2.5 cm in diameter, next to the head of the pancreas with turbulent flow inside.

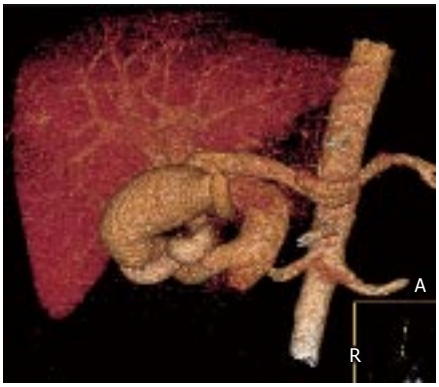


Figure 2 MDCT 3D reconstruction showed the presence of a large aneurysm filled with a dilated gastroduodenal artery and draining into the superior mesenteric vein.

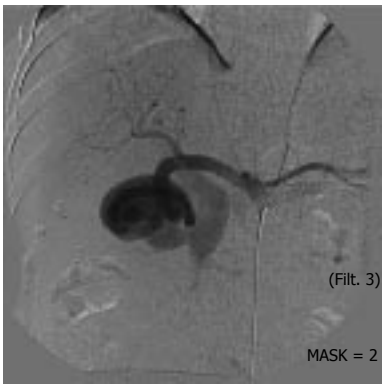


Figure 3 Celiac axis arteriogram confirmed a large aneurysm of gastro-duodenal artery and a fistulous communication with the main portal vein.

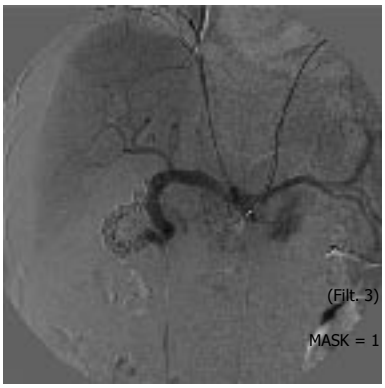


Figure 4 Arteriogram performed after coils embolization showed complete occlusion of the fistula.

(MIP) and Volume Rendering (VR) 3D angiography reconstructions clearly confirmed the presence of a large aneurysm filled by a dilated gastroduodenal artery, draining into the superior mesenteric vein (Figure 2). Other CT findings were normal liver, presence of esophageal varices and splenomegaly (volume of 910 mL). The HVPG was normal (5 mmHg), confirming the extrahepatic origin of portal hypertension. The diagnosis of extrahepatic portal hypertension secondary to arteroportal fistula was established. However, the patient refused the percutaneous arterial embolization of the fistula.

Seven months later a clinical examination revealed the presence of ascites. The MRI confirmed the presence of free fluid into the abdomen, bilateral pleural effusion and no changes in the aneurysm. The percutaneous embolization was then accepted. The initial angiogram of the celiac axis confirmed the large aneurysm of the gastro-duodenal artery and a fistulous communication with the main portal vein (Figure 3). After selective catheterization of the aneurysm, several metallic coils (8 mm in diameter) were placed in both the aneurysm and the fistula. The post-procedure arteriogram showed the complete occlusion of the fistula (Figure 4).

One month later, the endoscopy demonstrated the absence of esophageal varices. Three months later, a CT scan showed a complete thrombosis of the arteroportal fistula, the absence of ascites and pleural effusion, and a significant reduction of the spleen volume (689 mL). Now, the patient is free of symptoms with normal liver function tests: AST 34 U/L, ALT 32 U/L, serum bilirubin 0.8 mg/dL, albumin 3.9 g/dL, PT 98%, INR 0.8, and platelets count 22 × 10^3 μL.

DISCUSSION

The arteroportal fistula is a rare disorder of hepatic vasculature characterized by anomalous communication between the arteries and portal venous system. Extrahepatic arteroportal fistulas are less common than the intrahepatic ones. The most common causes of arteroportal venous fistula are traumas, iatrogenic damage and congenital vascular malformations[1]. In our patient there was no history of traumas or other vascular malformations, suggesting that the previous gastrectomy might be the most likely cause of arterioportal fistula. It is known that this vascular disorder may cause portal hypertension in non-cirrhotic liver[2,3], due to an increased arterial blood flow. In addition, the nodular regenerative hyperplasia and hepatoportal sclerosis with fibrosis may develop during the follow-up period and worsen portal hypertension[3].

In our case, the arteroportal venous fistula was well detected by Doppler ultrasonography, and confirmed by computed tomography and magnetic resonance angiography.

Severe portal hypertension with large esophageal varices, ascites and pleural effusion was maintained by the increased portal venous inflow. This is confirmed by a

normal value of HVPG that rules out the hepatic origin of portal hypertension. The arteroportal venous fistula can be treated with percutaneous arterial occlusion[4]. Notably, esophageal varices and ascites disappeared after the embolization, which further confirmed the role of increased portal blood inflow.

In conclusion, arteroportal fistula is a rare cause of portal hypertension in non-cirrhotic liver and may cause variceal bleeding and ascites. Imaging studies are useful to detect it and the HVPG measurement is useful in confirming the extrahepatic origin of portal hypertension. The percutaneous arterial embolization is an effective therapy for these patients.

REFERENCES

1 **Tomczak R**, Helmberger T, Görich J, Schütz A, Merkle E, Brambs HJ, Rieber A. [Abdominal arteriovenous and arterio-portal fistulas: etiology, diagnosis, therapeutic possibilities]. *Z Gastroenterol* 1997; **35**: 555-562

2 **Kang TC**, Lee HC, Yang FS, Wang NL. Idiopathic hepatic arterio-portal fistula: report of one case. *Acta Paediatr Taiwan* 2002; **43**: 102-105

3 **Aithal GP**, Alabdi BJ, Rose JD, James OF, Hudson M. Portal hypertension secondary to arterio-portal fistulae: two unusual cases. *Liver* 1999; **19**: 343-347

4 **Baumann UA**, Tillmann U, Frei J, Fehr HF. [Arterial aneurysm of the liver with arterio-portal fistula: treatment using embolization]. *Schweiz Med Wochenschr* 1984; **114**: 451-453

S- Editor Wang J **L- Editor** Ma JY **E- Editor** Bai SH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5565-5568
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Successful outcome after combined chemotherapeutic and surgical management in a case of esophageal cancer with breast and brain relapse

Davide Adriano Santeufemia, Gianfranca Piredda, Giovanni Maria Fadda, Paolo Cossu Rocca, Salvatore Costantino, Giovanni Sanna, Maria Giuseppa Sarobba, Maria Antonietta Pinna, Carlo Putzu, Antonio Farris

Davide Adriano Santeufemia, Gianfranca Piredda, Giovanni Maria Fadda, Giovanni Sanna, Maria Giuseppa Sarobba, Maria Antonietta Pinna, Carlo Putzu, Antonio Farris, Department of Medical Oncology, University of Sassari, Sardinia, Italy
Paolo Cossu Rocca, Institute of Pathological Anatomy, University of Sassari, Sardinia, Italy
Salvatore Costantino, Institute of Radiology, University of Sassari, Sardinia, Italy
Correspondence to: Professor Antonio Farris, Chair of Medical Oncology, University of Sassari, Viale San Pietro 8, 07100, Sassari, Sardinia, Italy. afarris@uniss.it
Telephone: +39-79-228382
Received: 2006-05-29 Accepted: 2006-06-16

Abstract

Esophageal cancer (EC) is a highly lethal disease. Approximately 50% of patients present with metastatic EC and most patients with localized EC will have local recurrence or develop metastases, despite potentially curative local therapy. The most common sites of distant recurrence are represented by lung, liver and bone while brain and breast metastases are rare. Usually patients with advanced disease are not treated aggressively and their median survival is six months. We report a woman patient who developed breast and brain metastases after curative surgery. We treated her with a highly aggressive chemotherapeutic and surgical combination resulting in a complete remission of the disease even after 11-year follow-up. We think that in super selected patients with more than one metastasis, when functional status is good and metastases are technically resectable, a surgical excision may be considered as a salvage option and chemotherapy should be delivered to allow a systemic control.

Key words: Esophageal cancer; Breast and brain metastases; Combined chemotherapeutic and surgical treatment

Santeufemia DA, Piredda G, Fadda GM, Cossu Rocca P, Costantino S, Sanna G, Sarobba MG, Pinna MA, Putzu C, Farris A. Successful outcome after combined chemotherapeutic and surgical management in a case of esophageal cancer with breast and brain relapse. *World J Gastroenterol* 2006; 12(34): 5565-5568

http://www.wjgnet.com/1007-9327/12/5565.asp

INTRODUCTION

Esophageal cancer (EC) is a highly lethal disease, with an estimated annual incidence of 14 550 new cases and 13 770 related deaths in 2006 in the USA[1]. Approximately 50% of patients present with metastatic disease and most patients with localized EC will have local recurrence or develop metastases, despite potentially curative local therapy[2].

The most common sites of distant recurrence are represented by lung, liver and bone[3] while brain and breast metastases are rare. Brain metastases have been reported only in 3.6% of all resected patients[4] but their incidence in all patients with EC is higher[5].

Extra mammary malignancies rarely metastasize to the breast, usually in patients with disseminated neoplasms[6] and according to literature data, only few cases of breast metastases from EC have been reported so far[7-9].

The case presented here is a woman with EC who underwent surgery and later developed synchronous solitary breast and brain metastases. She showed uncommon sites of recurrence of the disease. Generally, patients with EC are not treated aggressively in the presence of advanced diseases. Nevertheless, we performed a highly combined aggressive chemotherapeutic and surgical approach resulting in a complete remission of the disease even after 11-year follow-up. Together with the clinical case description, considering the rarity of the event, we discussed the features connected with the diagnosis and management of such an uncommon presentation of the metastatic disease.

CASE REPORT

In June 1995 a 51-year-old white woman patient with EC was admitted to our hospital. She complained of severe dysphagia and dyspeptic disorders associated with a weight loss. Her history revealed recent onset of dysphagia,

Figure 1 Barium swallow shows the presence of a stenotic trait in the middle thoracic esophagus.

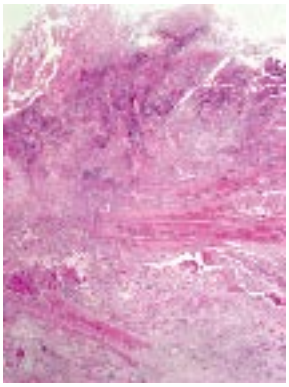


Figure 2 Esophageal histological sample (haematoxylin-eosin stain 20 x) shows the presence of squamous cell carcinoma with involvement of muscular tunic.

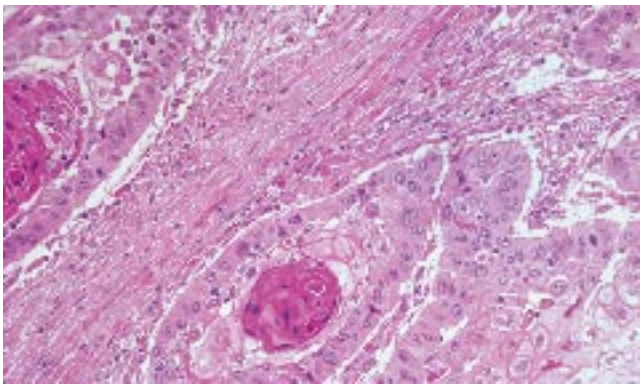


Figure 3 A squamous pearl at a higher magnification (haematoxylin-eosin stain 200 x).

present at first due to solid foods and then due to fluids as well. The difficult food intake caused a subsequent 5 kg weight loss during the five months prior to our observation. Since she had no other specific complaints, we decided to submit our patient to instrumental checks.

Barium swallow showed the presence of a 4 cm stenotic trait in the middle thoracic esophagus (Figure 1) and a next esophagogastroduodenoscopy revealed a vegetant mass in the esophageal lumen causing the stenosis. A biopsy of the mass was performed and histologic examination revealed a squamous esophageal carcinoma (Figures 2 and 3). The tumor marker values were increased and carbohydrate antigen 19-9 (CA19-9) was 250 U/mL (normal < 40 U/mL) while carcinoembryonic antigen (CEA) was 24 μg/L (normal < 3 μg/L). Other laboratory findings were normal.

Figure 4 Mammography shows a well-defined nodule without calcification.

Total body CT scan, abdominal ultrasonography and bone scintigraphy revealed that the disease was at stage IIa, we therefore decided to treat the patient surgically. In July 1995, subtotal esophagectomy was performed with dissection of lymph nodes and retrosternal reconstruction using tubulized gastric stump. Surgical operation was radical and the lymph nodes were negative for metastases. Histological examination confirmed the presence of squamous carcinoma and pathological stage of the disease was T_2 N_o M_o. The woman underwent periodical clinical checks.

In November 1995, the patient noted the presence of an approximately 3 cm × 3 cm nodule in the upper lateral quadrant of the left breast. At clinical examination, it appeared as a hard mass not fixed to the surrounding structures. The tumor marker carbohydrate antigen 15-3 (CA15-3) was normal. A mammography confirmed the presence of the nodule (Figure 4) and fine-needle aspiration cytology under ultrasound guidance was performed, indicating the presence of squamous carcinoma cells.

Total body CT scan requested for restaging showed also a 1 cm × 1 cm focal lesion in the frontal right lobe of the brain. Therefore, we treated the woman with a palliative chemotherapy schedule based on cisplatin (25 mg/m^2 iv a day) from d 1 to d 5, and 5-fluorouracil (1000 mg/m^2 iv a day) from day 1 to day 5 by continuous infusion every four weeks[10]. After the first cycle administration we observed remarkable reduction of the breast metastasis at clinical examination.

Taking into account either the palliative medical treatment or her relatively young age and good general conditions, we decided to perform surgical excision of both metastases followed by chemotherapy. In the following ten days, either tumorectomy or brain metastasectomy of the breast lesion was performed. Histological examination (Figures 5 and 6) revealed the presence of esophageal metastatic tumor. No surgical complication occurred. One month later, we resumed chemotherapy and further five courses of treatment were delivered.

In the following time the patient began to start again her daily activities and underwent periodical clinical and instrumental checks, which were always negative. The woman enjoyed good health after eleven years.

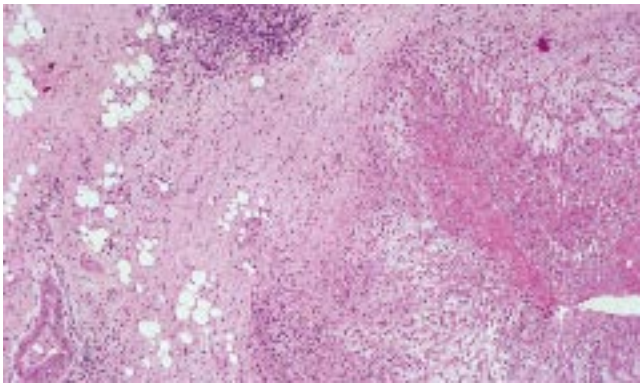


Figure 5 The right side of breast histological sample shows an infiltranting squamous carcinoma's focus with peritumoral inflammation while the left side shows a normal mammary duct section (haematoxylin-eosin stain 40 x).

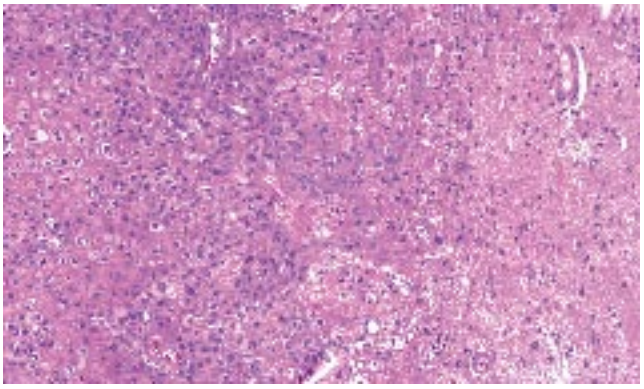


Figure 6 Squamous carcinoma infiltrating the cerebral parenchyma (haematoxylin-eosin stain 100 x).

DISCUSSION

EC is treatable but rarely curable. Patients with metastatic EC have a median survival time of six months[11]. Moreover the reported 5-year survival rate ranges from 20% to 36% after intentionally curative surgery[12] due to a high rate of either local or distant recurrence. Distant metastasis rate is reported to be 26% within 20 mo after radical surgery[13]. Early metastatic relapse after complete resection of any apparently localized primary tumor indicates that micrometastatic tumor cell spread at the time of surgery is undetectable by current staging methods and routine histopathology[14].

Commonly EC metastasizes to the lungs, liver and bone[3] but rarely to the brain[4] and exceptionally to the breast[7-9]. We observed an unusual metastatic pattern with both solitary breast and brain single metastases in our patient. Breast metastases from non-mammary malignancies are very rare and their incidence ranges from 0.5% to 5.1% of all breast tumors[7]. They usually occur in the upper outer quadrant[6] and their prognosis is poor[8,9].

Clinical and radiological aspects of secondary breast tumors are heterogeneous, though in most cases they appear as a solitary palpable, usually mobile mass. Mammographic finding consists of a round, dense and well-circumscribed mass, without spiculature, microcalcification or skin thickening. Furthermore, growth is usually rapid[15]. However, differential diagnosis is needed for correct treatment choice and it is reasonable to obtain a histological or cytological sample of the lesion.

The presence of metastatic lesions in any patient is an indication for a systemic treatment of primary cancer. Anyhow it has been reported that curative surgery for breast metastasis is a correct choice of treatment if metastases in other sites are controlled by other modalities[7].

For the case reported here, we made a disease restaging which showed concomitant presence of a brain metastasis because of the detection of her breast metastasis. This feature represents a further worsening of the prognosis of our patient. As a matter of fact patients with brain metastases from EC have a median survival time of 3.9 mo[16]. However it has been reported that an intensive brain tumor treatment may result in a longer survival time of selected patients with good Karnofsky performance status (KPS)[17] after excision of a single brain metastasis from EC[18]. Even the presence of multiple or recurrent brain metastases does not automatically controindicate surgery because it can prolong survival and enhance the quality of life[19]. Currently surgery followed by whole brain radiation therapy (WBRT) is considered the standard treatment[20] for a single brain metastasis.

For the case reported here, we began chemotherapy in order to obtain a systemic control of the disease. Since we observed a remarkable reduction of the breast lesion after only one course of chemotherapy, we modified our treatment plan. Since the patient was relatively young and in good functional status, we decided to perform radical excision of her metastatic EC followed by chemotherapy. Brain radiotherapy was not performed. This combined aggressive chemotherapeutic and surgical approach resulted in a complete remission of the disease even after 11-year follow-up.

Generally, the prognosis of patients with recurrent EC is poor, but in some cases surgical resection, chemotherapy or radiotherapy has been proven effective[21].

Our experience suggests that when functional status is good and metastases are technically resectable, surgical excision may be considered in selected patients with more than one metastasis and chemotherapy should be delivered to allow a systemic control.

REFERENCES

1 **Jemal A**, Siegel R, Ward E, Murray T, Xu J, Smigal C, Thun MJ. Cancer statistics, 2006. *CA Cancer J Clin* 2006; **56**: 106-130

2 **Enzinger PC**, Ilson DH, Kelsen DP. Chemotherapy in esophageal cancer. *Semin Oncol* 1999; **26**: 12-20

3 **Posner MC**, Forastiere AA, Minsky BD. Cancer of the esophagus. In: De Vita VT Jr, Hellman S, Rosenberg SA eds. Cancer Principles & Practice of Oncology. 7th ed. Philadelphia: Lippincott Williams & Wilkins, 2005: 861-909

4 **Gabrielsen TO**, Eldevik OP, Orringer MB, Marshall BL. Esophageal carcinoma metastatic to the brain: clinical value and cost-effectiveness of routine enhanced head CT before esophagectomy. *AJNR Am J Neuroradiol* 1995; **16:** 1915-1921

5 **Mandard AM**, Chasle J, Marnay J, Villedieu B, Bianco C, Roussel A, Elie H, Vernhes JC. Autopsy findings in 111 cases of esophageal cancer. *Cancer* 1981; **48**: 329-335

6 **Toombs BD**, Kalisher L. Metastatic disease to the breast: clini-

cal, pathologic, and radiographic features. *AJR Am J Roentgenol* 1977; **129**: 673-676
7 **Shiraishi M**, Itoh T, Furuyama K, Yamasaki S, Shimada Y, Hosotani R, Nakashima Y, Imamura M. Case of metastatic breast cancer from esophageal cancer. *Dis Esophagus* 2001; **14**: 162-165
8 **Nielsen M**, Andersen JA, Henriksen FW, Kristensen PB, Lorentzen M, Ravn V, Schiødt T, Thorborg JV, Ornvold K. Metastases to the breast from extramammary carcinomas. *Acta Pathol Microbiol Scand A* 1981; **89**: 251-256
9 **Miyoshi K**, Fuchimoto S, Ohsaki T, Sakata T, Takeda I, Takahashi K, Ohkawa T, Murata T, Kuwada Y. A Case of Esophageal Squamous Cell Carcinoma Metastatic to the Breast. *Breast Cancer* 1999; **6**: 59-61
10 **Ajani JA**, Ryan B, Rich TA, McMurtrey M, Roth JA, DeCaro L, Levin B, Mountain C. Prolonged chemotherapy for localised squamous carcinoma of the oesophagus. *Eur J Cancer* 1992; **28A**: 880-884
11 **Albertsson M**. Chemoradiotherapy of esophageal cancer. *Acta Oncol* 2002; **41**: 118-123
12 **Lerut T**, De Leyn P, Coosemans W, Van Raemdonck D, Scheys I, LeSaffre E. Surgical strategies in esophageal carcinoma with emphasis on radical lymphadenectomy. *Ann Surg* 1992; **216**: 583-590
13 **Law SY**, Fok M, Wong J. Pattern of recurrence after oesophageal resection for cancer: clinical implications. *Br J Surg* 1996; **83**: 107-111
14 **Hosch SB**, Stoecklein NH, Pichlmeier U, Rehders A, Scheunemann P, Niendorf A, Knoefel WT, Izbicki JR. Esophageal cancer: the mode of lymphatic tumor cell spread and its prognostic significance. *J Clin Oncol* 2001; **19**: 1970-1975
15 **McIntosh IH**, Hooper AA, Millis RR, Greening WP. Metastatic carcinoma within the breast. *Clin Oncol* 1976; **2**: 393-401
16 **Ogawa K**, Toita T, Sueyama H, Fuwa N, Kakinohana Y, Kamata M, Adachi G, Saito A, Yoshii Y, Murayama S. Brain metastases from esophageal carcinoma: natural history, prognostic factors, and outcome. *Cancer* 2002; **94**: 759-764
17 **Khuntia D**, Sajja R, Chidel MA, Lee SY, Rice TW, Adelstein DJ, Carlson TP, Saxton JP, Barnett GH, Suh JH. Factors associated with improved survival in patients with brain metastases from esophageal cancer: a retrospective review. *Technol Cancer Res Treat* 2003; **2**: 267-272
18 **Kesler KA**, Hammoud ZT, Helft PR, Rieger KM, Pritz MB, Brown JW. Long-term survival after excision of a solitary esophageal cancer brain metastasis. *J Thorac Cardiovasc Surg* 2006; **131**: 497-498
19 **Lang FF**, Sawaya R. Surgical treatment of metastatic brain tumors. *Semin Surg Oncol* 1998; **14**: 53-63
20 **Fujimaki T**. Surgical treatment of brain metastasis. *Int J Clin Oncol* 2005; **10**: 74-80
21 **Saeki H**, Kawaguchi H, Araki K, Ohno S, Sugimachi K. [Treatment strategy for and clinical results in patients with recurrent esophageal cancer]. *Nihon Geka Gakkai Zasshi* 1999; **100**: 185-190

S- Editor Wang J **L- Editor** Wang XL **E- Editor** Ma WH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5569-5572
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Collision tumor of the rectum: A case report of metastatic gastric adenocarcinoma plus primary rectal adenocarcinoma

Young-Hoon Roh, Hyoun-Wook Lee, Min-Chan Kim, Kyeong-Woo Lee, Mee-Sook Roh

Young-Hoon Roh, Min-Chan Kim, Department of Surgery, Dong-A University College of Medicine, Busan 602-715, South Korea
Hyoun-Wook Lee, Mee-Sook Roh, Department of Pathology, Dong-A University College of Medicine, Busan 602-715, South Korea
Kyeong-Woo Lee, Department of Rehabilitation Medicine, Dong-A University College of Medicine, Busan 602-715, South Korea
Mee-Sook Roh, Medical Research Center for Cancer Molecular Therapy, Dong-A University College of Medicine, Busan 602-715, South Korea
Supported by the Korea Science and Engineering Foundation through the MRCCMT at Dong-A University
Correspondence to: Mee-Sook Roh, MD, PhD, Department of Pathology, Dong-A University College of Medicine 1, 3-ga, Dongdaeshin-dong, Seo-gu, Busan 602-715, Korea. msroh@dau.ac.kr
Telephone: +82-51-2402833 Fax: +82-51-2437396
Received: 2006-04-16 Accepted: 2006-05-22

Abstract

Collision tumors are thought to arise from the accidental meeting and interpenetration of two independent tumors. We report here a highly unusual case of a 61-year old man who had a unique tumor that was composed of a metastatic adenocarcinoma from the stomach to the rectum, which harbored a collision tumor of primary rectal adenocarcinoma. The clonalities of the two histologically distinct lesions of the rectal mass were confirmed by immunohistochemical and molecular analysis. Although histologic examination is the cornerstone in pathology, immunohistochemical and molecular analysis can provide evidence regarding whether tumors originate from the same clone or different clones. To the best of our knowledge, this is the first reported case of such an occurrence.

Key words: Collision tumor; Rectum; Clonality

Roh YH, Lee HW, Kim MC, Lee KW, Roh MS. Collision tumor of the rectum: A case report of metastatic gastric adenocarcinoma plus primary rectal adenocarcinoma. *World J Gastroenterol* 2006; 12(34): 5569-5572

http://www.wjgnet.com/1007-9327/12/5569.asp

INTRODUCTION

The term collision tumor refers to two coexisting, but independent tumors[1]. Malignant neoplasms originating from two or more distinct topographic organs may form a collision tumor. A possible explanation for this is field cancerization, which occurs due to long-term exposure to carcinogens, whereby multiple carcinogenic transformations give rise to genetically unrelated secondary primary tumors with independent mutations[2,3] and thus, the chance of tumor collision may be increased. However, there is no explanation for the occurrence of many collision tumors. As most diagnoses are made based on the histology alone, the question is whether histologic classification can accurately reflect the molecular findings in these tumors. If two tumors arise independently and are associated with coincidence only, the genetic alterations are expected to be different from each other because of the different tumor origins. We report here a rare collision tumor of the rectum that was composed of a rectal adenocarcinoma within the metastatic gastric adenocarcinoma. Furthermore, in an effort to find the molecular evidence both for the histologic diagnosis of this collision tumor and for the clonality of the two separate components, we characterized the molecular alterations of each tumor component by examining the microsatellite instability (MSI) and loss of heterozygosity (LOH), and performed an immunohistochemical analysis as well. The findings of this tumor represent an entity that has never been described at this location.

CASE REPORT

A 61-year old man presented with postprandial epigastric pain for 5 mo. Upper gastrointestinal endoscopy suggested an ulcerofungating tumor spreading from the gastric body and antrum to near the esophagogastric junction, and biopsy revealed a poorly differentiated adenocarcinoma. The patient underwent radical total gastrectomy for the gastric cancer with regional lymph node dissection. During the operation, the rectum showed a tumor that was considered to be a distant metastasis from the gastric cancer. So, low anterior resection of the rectum with regional lymph node dissection was also performed for the distant metastasis of the gastric carcinoma.

The resected rectum revealed a relatively ill-defined ulcerofungating mass measuring 7 cm × 6 cm. Sectioning

Table 1 Results of immunohistochemistry for primary rectal carcinoma component and both primary and metastatic carcinoma components of the stomach

Immunohisto -chemical markers	Antibody			Results		
				Rectal tumor		Primary stomach
	Source	Clone	Dilution	Primary rectum	Metastatic stomach	
CK7	DakoCytomation	OV-TL	1:200	Positive	Negative	Negative
CK20	DakoCytomation	Ks20.8	1:50	Positive	Positive	Positive
p53	DakoCytomation	DO-7	1:50	Positive	Positive	Positive
MUC2	Novocastra	Ccp58	1:500	Negative	Positive	Positive
MUC5AC	Novocastra	CLH2	1:500	Negative	Negative	Negative
CDX2	Novocastra	CDX2-88	1:100	Positive	Positive	Positive

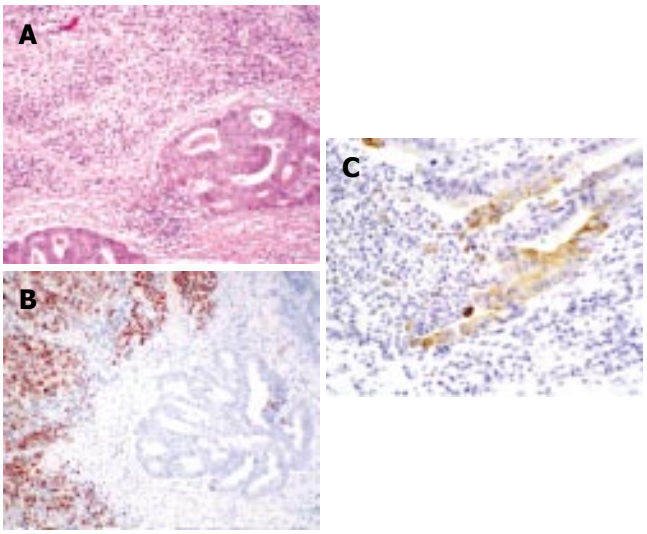


Figure 1 Histological features of rectal tumor showing the interface of a tubular adeonicarcinoma component of primary rectal cancer (right) and a poorly-differentiated adenocarcinoma component metastasized from the stomach (left) (hematoxylin and eosin; x 40) (**A**), immunohistochemical analysis showing positive MUC2 in metastatic gastric carcinoma and negative MUC2 in primary rectal carcinoma (x 200) (**B**), and negative CK7 in metastatic gastric carcinoma and positive CK7 in primary rectal carcinoma (x 200) (**C**).

revealed a whitish granular infiltrating tumor with extension into the perirectal soft tissue. Any regional differences of the tumor were not grossly identified. The resected stomach revealed a Borrmann type Ⅳ mass, measuring 14 cm × 14 cm, at nearly the entire gastric wall. Microscopically, the rectum showed a larger component of poorly differentiated adenocarcinoma with a focal signet-ring cell appearance involving the entire rectal wall. A smaller component of well-differentiated tubular adenocarcinoma invading into the perirectal soft tissue was noted within the poorly differentiated adenocarcinoma. Both components collided with each other with no intermingling at their interface (Figure 1A). The surgical margins were tumor-free. Multiple regional lymph nodes (n = 28) showed metastatic adenocarcinoma (n = 26), of which two revealed feature of well-differentiated tubular adenocarcinoma and 24 revealed poorly-differentiated adenocarcinoma. The stomach mass was an invasive, poorly-differentiated adenocarcinoma invading into the perigastric soft tissue and showing the same histologic features as the larger component of the rectal tumor. The well-differentiated tubular adenocarcinoma cells seen in the rectal wall were not found in the gastric wall. The proximal resection margin was tumor-involved, but the distal resection margin was tumor-free. Multiple regional lymph nodes (n = 60) showed poorly-differentiated metastatic adenocarcinoma (n = 47).

Immunohistochemical staining was performed to distinguish the two components of the rectal tumor. The characteristics of the antibodies used in this study and the results are presented in Table 1 as well as in Figure 1B and C. In summary, the primary gastric carcinoma as well as the metastatic gastric carcinoma in the rectum displayed both strong and diffuse staining for MUC2, but negative staining for cytokeratin 7 (CK7), whereas the primary rectal carcinoma component showed focal positive immunoreactivity for CK7, but negative staining for MUC2. The distribution of immunostaining was well correlated with the histologic distinction between metastatic gastric and primary rectal carcinoma components in the collision tumor.

The tissue of both tumors and their non-tumor counterparts were scraped from 10 μm-thick formalin-fixed, paraffin-embedded sections, and then genomic DNA was extracted using the DNeasy tissue kit (Qiagen, Hilden, Germany). DNA sample pairs were amplified using the microsatellite instability MSI/LOH starter kit (Applied Biosystems, Forster City, CA, USA). Genetic stability was analyzed using the Bethesda reference panel that includes BAT25, BAT26, D2S123, D5S346 and D17S250[4]. Both the primary and metastatic gastric carcinoma components as well as the primary rectal carcinoma component showed microsatellite stability (Figure 2). LOH analysis was carried out using 3 polymorphic microsatellite repeat markers including D2S123, D5S346 and D17S250. A value below 0.6 or above 1.6 was interpreted as evidence of LOH, whereas values between these figures were considered retention of heterozygosity. LOH was found in both primary and metastatic gastric carcinoma components, but not in primary rectal carcinoma component (Table 2). Eight months after surgery, the patient died of recurrent gastric cancer.

DISCUSSION

Collision tumor is considered as a double tumor showing a 'side by side' or 'one upon another' pattern. It can

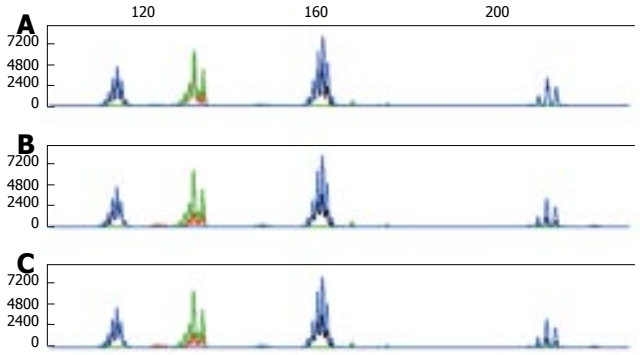


Figure 2 Microsatellite instability phenotype analysis showing no microsatellite instability in the primary rectal tumor (**A**), the metastatic gastric tumor (**B**) and the primary gastric tumor (**C**). Blue and green line: Normal tissue; Black and red line: Tumor tissue.

Table 2 Results of loss of heteozygosity analysis for primary rectal carcinoma component and both primary and metastatic carcinoma components of the stomach

Microsatellite marker	Chromosomal region	Tumor suppressor gene	Loss of heterozygosity		
			Rectal tumor		Primary stomach
			Primary rectum	Metastatic stomach	
D2S123	2p16	hMSH2	No (0.95)	No (0.93)	No (1.03)
D5S346	5q21	APC	No (1.31)	Yes (0.50)	Yes (0.54)
D17S250	17q11.2-12	P53	No (1.01)	Yes (0.41)	Yes (0.43)

occur within the same organ, or in adjacent organs, or in conjunction with systemic malignancy[5]. Several hypotheses have been suggested as the mechanisms for collision tumor. The simplest is that two primary tumors occur in continuity by an accidental "meeting". Two different tumors may also contiguously develop because the region is altered by the same carcinogenic stimuli. Another hypothesis is that the presence of the first tumor alters the microenvironment, making the development of the second adjacent tumor more likely[5]. In our case, because the primary rectal cancer occupied a smaller portion of the lesion within the larger portion of metastatic gastric cancer, suggesting that metastatic gastric carcinoma to the rectum probably makes some changes in microenvironment and metabolic condition of the rectum, in which a second primary rectal cancer may have developed in association with a previous metastatic focus rather than a metastasis harboring rectal adenocarcinoma. Therefore, we can postulate that substances produced by gastric adenocarcinoma stimulate the immediate adjacent mucosa to undergo increased proliferation and neoplastic transformation.

Collision tumor needs to be distinguished from composite tumor, which is characterized by two divergent lineages originating from the same neoplastic clonal proliferation[1], because different treatments are warranted depending on the type of collision tumor encountered[6]. The behavior of collision tumor depends on the individual elements. Definitive conclusion could not be drawn with regard to the prognosis of this type of collision tumor in our case. However, it is important to differentiate between a case with rectal carcinoma coincidentally having a metastatic gastric carcinoma component and a case with only a primary rectal carcinoma component because of the difference in prognosis. The prognosis of patients with only primary rectal carcinoma is determined by the staging at diagnosis and it is likely to be more favorable than that of patients with an additional metastatic gastric adenocarcinoma in the rectum.

Genetic analysis provides evidence regarding whether the tumors originate from the same clone or from different clones. The panels for each anatomically different site of tumor are then compared to identify the conserved and unique mutations. If the mutational profiles are predominantly unmatched, the diagnosis of a second primary tumor can be established. In an effort to determine the clonality of the two separate components in this patient's rectal tumor, we characterized the molecular alterations of each tumor component, by MSI and LOH analysis. It has been recently reported that the pattern of MSI findings is a useful tool in determining whether a patient has double primary tumors or a single clonal tumor with metastasis[7]. Kim *et al*[8] reported that high coincident MSI is observed in 17.7% of patients with colon and stomach cancers, suggesting that a genetic defect of mismatch repair deficiency may be responsible for a small subset of double primary cancers of the colorectum and stomach. In this case, we could not find any discriminating pattern of MSI in both primary and metastatic gastric carcinoma components, as well as in primary rectal carcinoma component. The clonal evolution of cancer can be followed up by using markers that identify LOH. However, carcinogenesis is most often not a single event, but rather the result of many mutations that accumulate over time. Blaker *et al*[9] reported that LOH patterns of primary colon cancer and metastatic tumors are different in about half of the cases. In our case, LOH analysis showed that the two components of rectal tumor were collision tumor.

The mucin gene family consists of at least nine MUC genes whose tissue distribution has mainly been studied with antibodies that recognize the core protein of different mucins. At immunohistochemical level, the expressed main mucin types are MUC1 for the intestinal type, MUC5AC for the diffuse type, and MUC2 for the mucinous type in gastric cancer[10]. MUC2 expression has been shown to be significantly lower in non-mucinous colorectal cancer[11]. Different expressions of various types of CK in tumors at different primary sites can be a clue to the origin of a neoplasm. It has been reported that carcinomas of the colon generally express CK20, whereas the CK7 expression is usually negative, and the expressions of CK7 and CK20 in carcinomas of the stomach have yielded more variable results[12]. However, carcinomas of a gastrointestinal origin exhibit overlapping and heterogeneous expressions of each mucin and also CK, and there is no definitively consistent immunoreactivity pattern. In this study, primary gastric carcinoma as well as metastatic gastric carcinoma in the rectum displayed strong and diffuse staining for MUC2, but negative staining for CK7, whereas the primary rectal carcinoma component showed focal

positive immunoreactivity for CK7, but negative staining for MUC2. The distribution of immunostaining was significantly correlated with the histologic distinction between metastatic gastric and primary rectal carcinoma components in collision tumor.

To the best of our knowledge, although one of the tumors with a significantly elevated risk is colorectal cancer after the diagnosis of stomach cancer, rectal collision tumor with primary rectal adenocarcinoma and metastatic gastric adenocarcinoma is an entity that has not been previously described at this location. Our immunohistochemical and molecular approach clearly demonstrates that the two components of adenocarcinoma of the rectum have a different clonality.

ACKNOWLEDGMENTS

The authors thank SH Jeong of the ISU ABXIS for her molecular technical assistance.

REFERENCES

1 **Willis RA**. Structure and growth of tumors. In: Willis RA, ed. Pathology of tumors. 4th ed. London: Butterworth, 1967: 138
2 **Eguchi K**, Yao T, Konomoto T, Hayashi K, Fujishima M, Tsuneyoshi M. Discordance of p53 mutations of synchronous colorectal carcinomas. *Mod Pathol* 2000; **13**: 131-139
3 **Piérard GE,** Fazaa B, Henry F, Kamoun MR, Piérard-Franchimont C. Collision of primary malignant neoplasms on the skin: the connection between malignant melanoma and basal cell carcinoma. *Dermatology* 1997; **194**: 378-379
4 **Umar A**, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Rüschoff J, Fishel R, Lindor NM, Burgart LJ, Hamelin R, Hamilton SR, Hiatt RA, Jass J, Lindblom A, Lynch HT, Peltomaki P, Ramsey SD, Rodriguez-Bigas MA, Vasen HF, Hawk ET, Barrett JC, Freedman AN, Srivastava S. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* 2004; **96**: 261-268
5 **Brandwein-Gensler M**, Urken M, Wang B. Collision tumor of the thyroid: a case report of metastatic liposarcoma plus papillary thyroid carcinoma. *Head Neck* 2004; **26**: 637-641
6 **Milne AN**, Carvalho R, van Rees BP, van Lanschot JJ, Offerhaus GJ, Weterman MA. Do collision tumors of the gastroesophageal junction exist? A molecular analysis. *Am J Surg Pathol* 2004; **28**: 1492-1498
7 **Tang M,** Pires Y, Schultz M, Duarte I, Gallegos M, Wistuba II. Microsatellite analysis of synchronous and metachronous tumors: a tool for double primary tumor and metastasis assessment. *Diagn Mol Pathol* 2003; **12**: 151-159
8 **Kim HS,** Cho NB, Yoo JH, Shin KH, Park JG, Kim YI, Kim WH. Microsatellite instability in double primary cancers of the colorectum and stomach. *Mod Pathol* 2001; **14**: 543-548
9 **Blaker H,** Graf M, Rieker RJ, Otto HF. Comparison of losses of heterozygosity and replication errors in primary colorectal carcinomas and corresponding liver metastases. *J Pathol* 1999; **188**: 258-262
10 **Lau SK**, Weiss LM, Chu PG. Differential expression of MUC1, MUC2, and MUC5AC in carcinomas of various sites: an immunohistochemical study. *Am J Clin Pathol* 2004; **122**: 61-69
11 **Losi L**, Scarselli A, Benatti P, Ponz de Leon M, Roncucci L, Pedroni M, Borghi F, Lamberti I, Rossi G, Marino M, Ponti G, Zangardi G, Menigatti M, Di Gregorio C. Relationship between MUC5AC and altered expression of MLH1 protein in mucinous and non-mucinous colorectal carcinomas. *Pathol Res Pract* 2004; **200**: 371-377
12 **Osborn M,** van Lessen G, Weber K, Klöppel G, Altmannsberger M. Differential diagnosis of gastrointestinal carcinomas by using monoclonal antibodies specific for individual keratin polypeptides. *Lab Invest* 1986; **55**: 497-504

S- Editor Wang J **L- Editor** Wang XL **E- Editor** Ma WH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5573-5576
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Mucosa-associated lymphoid tissue lymphoma of the transverse colon: A case report

Shigetoshi Matsuo, Yohei Mizuta, Tomayoshi Hayashi, Seiya Susumu, Ryuji Tsutsumi, Takashi Azuma, Satoshi Yamaguchi

Shigetoshi Matsuo, Seiya Susumu, Ryuji Tsutsumi, Takashi Azuma, Satoshi Yamaguchi, Department of Surgery, Nagasaki Prefectural Shimabara Hospital, Shimabara, Nagasaki, Japan
Yohei Mizuta, Second department of Internal medicine, Nagasaki University School of Medicine, Nagasaki, Japan
Tomayoshi Hayashi, Department of Pathology, Nagasaki Prefectural Shimabara Hospital, Shimabara, Nagasaki, Japan
Correspondence to: Dr. Shigetoshi Matsuo, Department of Surgery, Nagasaki Prefectural Shimabara Hospital, 7895 Shimokawajiri, Shimabara, Nagasaki 855-0861, Japan. shigetoshi-matsuo@pref.nagasaki.lg.jp
Telephone: +81-957-631145 Fax: +81-957-634864
Received: 2006-06-07 Accepted: 2006-07-07

Abstract

We herein present a case of a 75-year-old female with mucosa-associated lymphoid tissue (MALT) lymphoma of the transverse colon with the stage IE (Ann Arbor classification). Colonoscopy revealed the tumor's appearance as a IIa plus IIc-like early colon cancer as defined according to the macroscopic classification of the Japanese Research Society for Cancer of Colon, Rectum and Anus, measuring less than 2 cm in diameter. Histologically, the tumor was diagnosed as MALT lymphoma because of the presence of lymphoepithelial lesions consisting of diffuse proliferation of atypical lymphocytes and glandular destruction. The majority of these lymphocytes immunohistochemically stained for the B-lymphocyte marker. The patient first underwent *H pylori* eradication therapy with Lansap®. However, the tumor size gradually increased over the next 4 mo and the patient eventually underwent surgical resection. The operative procedure included a partial colectomy with dissection of the paracolic lymph nodes. The tumor measured 45 mm x 30 mm in diameter and histological examination showed that the lymphoma cells had infiltrated the muscle layer of the colon without nodal involvement. The patient has had no recurrence postoperatively without any chemotherapy.

Key words: Mucosa-associated lymphoid tissue; Malignant lymphoma

Matsuo S, Mizuta Y, Hayashi T, Susumu S, Tsutsumi R, Azuma T, Yamaguchi S. Mucosa-associated lymphoid tissue lymphoma of the transverse colon: A case report. *World J Gastroenterol* 2006; 12(34): 5573-5576

http://www.wjgnet.com/1007-9327/12/5573.asp

INTRODUCTION

The term mucosa-associated lymphoid tissue (MALT) lymphoma was first introduced by Isaacson and Wright[1] in 1983. This entity includes low-grade gastric B-cell lymphoma and immunoproliferative small intestinal disease. MALT lymphomas occur in a variety of extranodal organs, such as the gastrointestinal (GI) tract and the non-GI tract, in which the stomach is the most common site[2,3]. Since convincing evidence has been presented showing the relationship between *H pylori* and gastric MALT lymphoma, the therapeutic strategy has been altered for patients with gastric MALT lymphoma in the early stages[4-8]. In contrast, a treatment for colonic MALT lymphoma has not yet been established. In the present report, we describe a case of colonic MALT lymphoma which did not response to *H pylori* eradication treatment and, therefore, underwent a surgical resection, and also provide a literature review on this rare entity.

CASE REPORT

A 75-year-old female was admitted to Nagasaki Prefectural Shimabara Hospital for surgical treatment of MALT lymphoma of the transverse colon on June 21, 2005. The patient had shown a positive fecal occult blood test on January 20, 2005 without any clinical symptoms and signs. Her past histories included hypertension, diabetes mellitus, and cholecystolithiasis. The results of complete blood counts, blood chemistries and tumor markers, such as carcinoembryonic antigen, were all within the normal limits. Colonoscopy revealed the tumor's appearance as IIa plus IIc-like early colon cancer[9], measuring less than 2 cm in diameter, in the transverse colon (Figure 1). Biopsy specimens histologically showed lymphoepithelial lesions with diffuse proliferation of atypical small lymphocytes and some glandular destruction. These lymphocytes immunohistochemically showed diffusely positive staining for L-26 (Figure 2) and bcl-2, but negative staining for CD3, CD5, CD10, CD79a, UCHL-1 and cyclin D1. These findings were compatible with MALT lymphoma of the colon. Barium enema showed a flat-elevated lesion in the transverse colon (Figure 3). Abdominal and chest CT demonstrated neither abnormal lesions nor

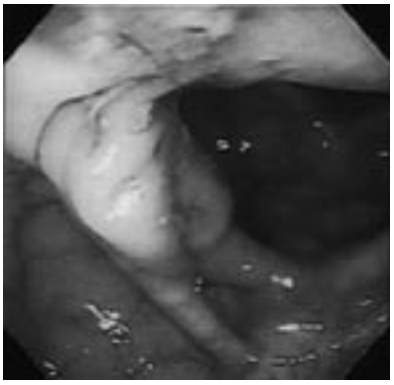


Figure 1 Colonofiberscopy showing the tumor's appearance as a IIa plus IIc-like early colon cancer.

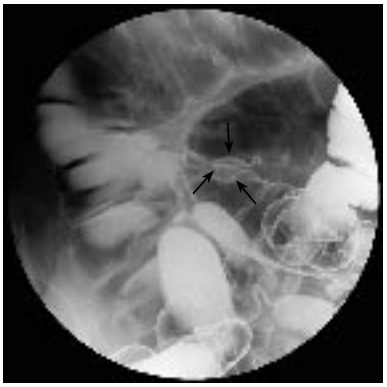


Figure 3 Barium enema showing a flat and well-circumscribed tumor in the transverse colon (arrows).

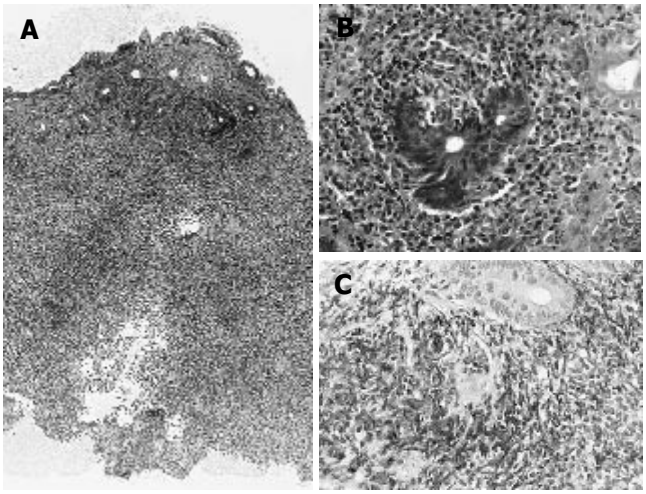


Figure 2 Biopsy specimens histologically showing diffuse proliferation of atypical small lymphocytes in the mucosal layer (**A**: x 40 magnification, HE) and glandular destruction (**B**: x 200 magnification, HE). These lymphocytes immunohistochemically showing diffusely positive staining for L-26 (**C**; x 200 magnification, ABC method).

Figure 4 Resected specimens showing a flat-elevated tumor with slight depression, measuring 45 mm x 30 mm in diameter.

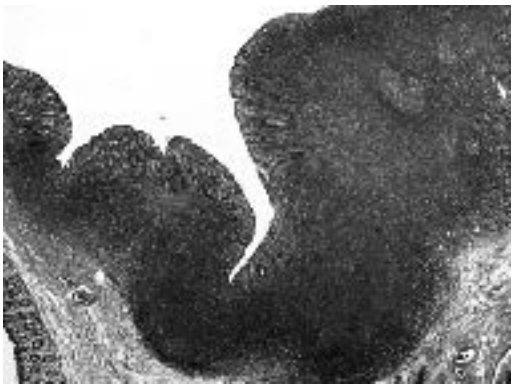


Figure 5 The lymphoma cells mainly infiltrated into the mucosal and submucosal layers, and partly infiltrated into the muscular layer of the colon (x 20 magnification, HE).

enlargement of lymph node. According to the Ann Arbor classification, this MALT lymphoma belonged to the stage IE. The patient first underwent *H pylori* eradication therapy with Lansap® because of positive reaction to a urea breath test (UBT). However, over the course of 4 mo, the tumor gradually increased in size, although *H pylori* were eradicated. The patient was considered to be a non-responder to eradication therapy, and was indicated for surgical resection. A partial colectomy with dissection of the paracolic lymph nodes was performed on June 23, 2005 (Figure 4). The tumor grossly appeared to be a IIa plus IIc-like early colon cancer, measuring 45 mm × 30 mm in diameter. Resected specimens were histologically and immunohistochemically reconfirmed to be MALT lymphoma without nodal involvement. The lymphoma cells infiltrated mainly into the mucosa and submucosal layer, and partly infiltrated into the muscular layer of the colon (Figure 5). The patient has had no recurrence postoperatively without any chemotherapy.

DISCUSSION

MALT lymphoma is defined as extra-nodal marginal zone B-cell lymphoma of MALT type in peripheral B-cell lymphoma according to morphologic features, immunophenotype, genetic features, postulated normal counterpart, and clinical features[10,11].

H pylori eradication therapy is currently widely recognized as an initial therapy in cases with low-grade (stage I) gastric MALT lymphoma[12-14]. In contrast, it has not yet been clarified whether *H pylori* eradication, or chemotherapy, or surgery should be performed in colonic MALT lymphoma compared with gastric MALT lymphoma, because the colorectal MALT lymphomas are rare. The individual clinical details of colorectal MALT lymphoma are summarized in Table 1[15-27]. Some reports have

Table 1 Colorectal MALT lymphoma in the English literatures

Author	Yr	Age/Sex	Location/Size	Symptoms/Signs	*H pylori*	Treatment	Outcome
Schmid[15]	1994	65/M	S/2.5 cm	ND	ND	Polypectomy	NED for 9 mo
	1994	47/M	T/1.5 cm	ND	ND	Polypectomy and chemotherapy	NED for 24 mo
	1994	64/M	T/1.5 cm	ND	ND	Left hemicolectomy	Died, 7 d (Cardiac failure)
Matsumoto[16]	1997	72/F	R/ND	Rectal bleeding	+	Eradication	NED for 12 wk
Yasui[17]	1999	76/M	C/30 × 15 mm	Fecal occult blood	ND	Partial resection[2]	ND
Orita[18]	1999	64/F	R/3.4 × 4.8 cm	Fecal occult blood	ND	Abdominoperineal resection	NED for 35 mo
Inoue[19]	1999	62/F	R/ND	Hematochezia	-	Eradication	NED for 53 wk
Raderer[20]	2000	67/M	D[1]/1.5 cm	None	+	Eradication	NED for 4 mo
Hasegawa[21]	2000	72/F	S/3.0 × 1.5 cm	Abdominal pain, fever	ND	Sigmoidectomy	ND
Yoshimura[22]	2002	74/M	T/8 × 4 cm	Fecal occult blood	ND	Chemotherapy	NED for 20 mo
Nakase[23]	2002	66/F	D, S, R/ND	Hematochezia	-	Eradication	NED for 1.5 yr
	2002	33/F	R/ND	Fever, hematochezia	-	Eradication	NED for 10 mo
	2002	62/F	R/ND	Hematochezia	-	Eradication	NED for 6 mo
Hisabe[24]	2002	70/F	R/1.5 cm	Abdominal discomfort	-	Eradication	NED for 20 mo
Takada[25]	2003	44/M	C/1.1 × 0.9 cm	Fecal occult blood	-	Partial resection[2]	ND
Lee[26]	2005	47/M	R/ND	Tenesmus, mucoid sool	-	Chemotherapy and radiation	NED for 3 mo
Kikuchi[27]	2005	71/M	R/3.5 cm	Fecal occult blood	-	Eradication[3]	NED for 12 mo
	2005	80/F	C, R/2.5 cm	Anal bleeding	-	Eradication	NED for 6 mo
	2005	70/F	R/1.5 cm	Abdominal discomfort	-	Eradication	NED for 20 mo
Our case	2006	75/F	T/4.5 × 3.5 cm	Fecal occult blood	+	Partial resection after eradication	NED for 12 mo

Location; R: Rectum, S: Sigmoid colon; D: Descending colon; T: Transverse colon; C: Cecum; ND: Not described; NED: No evidence of disease. [1]This patient had MALT lymphomas in the stomach and the descending colon, simultaneously. [2]These patients performed laparoscopy-assisted colon resection. [3]This patient has received repeated eradication.

described the successful regression of colorectal MALT lymphoma by means of eradication therapy in *H pylori*-positive patients[16,20]. Even in cases with colorectal MALT lymphoma negative for *H pylori*, the regression of the tumor was also recognized as a result of *H pylori* eradication therapy[19,23,24,27]. In prospective studies of gastric MALT lymphoma, eradication therapy was not effective in patients negative for *H pylori*[28,29]. Grunberger *et al*[30] reported that antibiotic eradication therapy was not effective in patients infected with *H pylori* suffering from extra-gastric MALT lymphoma. Therefore, they suggested that *H pylori* did not play a role in the development of extra-gastric MALT lymphomas. Similarly, antibiotic eradication therapy was not effective in our present case. These results may suggest that colorectal MALT lymphomas are not directly related to *H pylori* infection, while gastric MALT lymphomas are strongly associated with *H pylori* infection. In the future, a definite pathogenesis of colorectal MALT lymphoma should be clarified when cases of colorectal MALT lymphomas have accumulated. As a speculation, colorectal MALT lymphomas may be caused by unknown antibiotic-sensitive microorganisms other than *H pylori*, although that is not clear. In gastric MALT lymphoma, indeed, *Helicobacter heilmannii*-associated MALT lymphoma other than *H pylori* has been reported to be completely regressed by eradication therapy[31].

Surgical resection is mandatory when a colorectal MALT lymphoma does not respond to eradication therapy or chemotherapy, and it is localized without dissemination.

In conclusion, the present case with colonic MALT lymphoma eventually underwent surgical resection of the colon after the failure of eradication therapy. Surgical intervention is now the procedure of choice for colorectal MALT lymphoma because its pathogenesis and therapeutic strategy have not yet been established.

REFERENCES

1 **Isaacson P**, Wright DH. Malignant lymphoma of mucosa-associated lymphoid tissue. A distinctive type of B-cell lymphoma. *Cancer* 1983; **52**: 1410-1416

2 **Thieblemont** C, Bastion Y, Berger F, Rieux C, Salles G, Dumontet C, Felman P, Coiffier B. Mucosa-associated lymphoid tissue gastrointestinal and nongastrointestinal lymphoma behavior: analysis of 108 patients. *J Clin Oncol* 1997; **15**: 1624-1630

3 **Thieblemont C**, Berger F, Dumontet C, Moullet I, Bouafia F, Felman P, Salles G, Coiffier B. Mucosa-associated lymphoid tissue lymphoma is a disseminated disease in one third of 158 patients analyzed. *Blood* 2000; **95**: 802-806

4 **Wyatt JI**, Rathbone BJ. Immune response of the gastric mucosa to Campylobacter pylori. *Scand J Gastroenterol Suppl* 1988; **142**: 44-49

5 **Stolte M**, Eidt S. Lymphoid follicles in antral mucosa: immune response to Campylobacter pylori? *J Clin Pathol* 1989; **42**: 1269-1271

6 **Isaacson PG**. Gastric MALT lymphoma: from concept to cure. *Ann Oncol* 1999; **10**: 637-645

7 **Neubauer A**, Thiede C, Morgner A, Alpen B, Ritter M, Neubauer B, Wündisch T, Ehninger G, Stolte M, Bayerdörffer E. Cure of Helicobacter pylori infection and duration of remission of low-grade gastric mucosa-associated lymphoid tissue lymphoma. *J Natl Cancer Inst* 1997; **89**: 1350-1355

8 **Montalban C**, Santon A, Boixeda D, Bellas C. Regression of gastric high grade mucosa associated lymphoid tissue (MALT) lymphoma after Helicobacter pylori eradication. *Gut* 2001; **49**: 584-587

9 Japanese Research Society for Cancer of the Colon, Rectum and Anus. General rules for clinical and pathological studies on cancer of colon, rectum and anus (6th ed). Tokyo: Kinbara Shuppan, 1998: 32-36

10 **Harris NL**, Jaffe ES, Stein H, Banks PM, Chan JK, Cleary ML, Delsol G, De Wolf-Peeters C, Falini B, Gatter KC. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994; **84**: 1361-1392

11 **Jaffe ES**, Harris NL, Diebold J, Muller-Hermelink HK. World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues. A progress report. *Am J*

Clin Pathol 1999; **111**: S8-12
12 **Wotherspoon AC**, Ortiz-Hidalgo C, Falzon MR, Isaacson PG. Helicobacter pylori-associated gastritis and primary B-cell gastric lymphoma. *Lancet* 1991; **338**: 1175-1176
13 **Wotherspoon AC**, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, Isaacson PG. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of Helicobacter pylori. *Lancet* 1993; **342**: 575-577
14 **Fischbach W**, Goebeler-Kolve ME, Dragosics B, Greiner A, Stolte M. Long term outcome of patients with gastric marginal zone B cell lymphoma of mucosa associated lymphoid tissue (MALT) following exclusive Helicobacter pylori eradication therapy: experience from a large prospective series. *Gut* 2004; **53**: 34-37
15 **Schmid C**, Vazquez JJ, Diss TC, Isaacson PG. Primary B-cell mucosa-associated lymphoid tissue lymphoma presenting as a solitary colorectal polyp. *Histopathology* 1994; **24**: 357-362
16 **Matsumoto T**, Iida M, Shimizu M. Regression of mucosa-associated lymphoid-tissue lymphoma of rectum after eradication of Helicobacter pylori. *Lancet* 1997; **350**: 115-116
17 **Yasui N**, Watanabe M, Iwao Y, Sakaguchi AA, Tahara T, Eramoto T, Mukai M, Hibi T, Kitajima M. Laparoscopically assisted bowel resection for primary mucosa-associated lymphoid tissue lymphoma of the cecum. *Surg Laparosc Endosc Percutan Tech* 1999; **9**: 156-159
18 **Orita M**, Yamashita K, Okino M, Enoki T, Noshima S, Morita N, Esato K. A case of MALT (mucosa-associated lymphoid tissue) lymphoma occurring in the rectum. *Hepatogastroenterology* 1999; **46**: 2352-2354
19 **Inoue F**, Chiba T. Regression of MALT lymphoma of the rectum after anti-H. pylori therapy in a patient negative for H. pylori. *Gastroenterology* 1999; **117**: 514-515
20 **Raderer M**, Pfeffel F, Pohl G, Mannhalter C, Valencak J, Chott A. Regression of colonic low grade B cell lymphoma of the mucosa associated lymphoid tissue type after eradication of Helicobacter pylori. *Gut* 2000; **46**: 133-135
21 **Hasegawa N**, Kato K, Yamada K, Morita K, Kuroiwa M, Ito H, Kamioka T, Matsuura T, Sato M, Ono K, Suzuki Y, Goto H. Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) of the sigmoid colon. *Gastrointest Endosc* 2000; **52**: 802-804
22 **Yoshimura D**, Maruoka A, Maekawa S, Yao T, Harada N, Nawata H. Transverse colon MALT lymphoma with nodal metastasis. *Gastrointest Endosc* 2002; **55**: 238-239
23 **Nakase H**, Okazaki K, Ohana M, Ikeda K, Uchida K, Uose S, Itoh T, Iwano M, Watanabe N, Yazumi S, Kawanami C, Inoue F, Chiba T. The possible involvement of micro-organisms other than Helicobacter pylori in the development of rectal MALT lymphoma in H. pylori-negative patients. *Endoscopy* 2002; **34**: 343-346
24 **Hisabe T**, Imamura K, Furukawa K, Tsuda S, Matsui T, Yao T, Kanda M, Ohshima K, Kikuchi M. Regression of CD5-positive and Helicobacter pylori-negative mucosa-associated lymphoid tissue lymphoma of the rectum after administration of antibiotics: report of a case. *Dis Colon Rectum* 2002; **45**: 1267-1270
25 **Takada M**, Ichihara T, Fukumoto S, Nomura H, Kuroda Y. Laparoscopy-assisted colon resection for mucosa-associated lymphoid tissue (MALT) lymphoma in the cecum. *Hepatogastroenterology* 2003; **50**: 1003-1005
26 **Lee YG**, Lee S, Han SW, Lee JS. A case of multiple mucosa-associated lymphoid tissue (MALT) lymphoma of the colon identified as simple mucosal discoloration. *J Korean Med Sci* 2005; **20**: 325-328
27 **Kikuchi Y**, Matsui T, Hisabe T, Wada Y, Hoashi T, Tsuda S, Yao T, Iwashita A, Imamura K. Deep infiltrative low-grade MALT (mucosal-associated lymphoid tissue) colonic lymphomas that regressed as a result of antibiotic administration: endoscopic ultrasound evaluation. *J Gastroenterol* 2005; **40**: 843-847
28 **Steinbach G**, Ford R, Glober G, Sample D, Hagemeister FB, Lynch PM, McLaughlin PW, Rodriguez MA, Romaguera JE, Sarris AH, Younes A, Luthra R, Manning JT, Johnson CM, Lahoti S, Shen Y, Lee JE, Winn RJ, Genta RM, Graham DY, Cabanillas FF. Antibiotic treatment of gastric lymphoma of mucosa-associated lymphoid tissue. An uncontrolled trial. *Ann Intern Med* 1999; **131**: 88-95
29 **Ruskoné-Fourmestraux A**, Lavergne A, Aegerter PH, Megraud F, Palazzo L, de Mascarel A, Molina T, Rambaud JL. Predictive factors for regression of gastric MALT lymphoma after anti-Helicobacter pylori treatment. *Gut* 2001; **48**: 297-303
30 **Grünberger B,** Wöhrer S, Streubel B, Formanek M, Petkov V, Puespoek A, Haefner M, Hejna M, Jaeger U, Chott A, Raderer M. Antibiotic treatment is not effective in patients infected with Helicobacter pylori suffering from extragastric MALT lymphoma. *J Clin Oncol* 2006; **24**: 1370-1375
31 **Morgner** A, Lehn N, Andersen LP, Thiede C, Bennedsen M, Trebesius K, Neubauer B, Neubauer A, Stolte M, Bayerdörffer E. Helicobacter heilmannii-associated primary gastric low-grade MALT lymphoma: complete remission after curing the infection. *Gastroenterology* 2000; **118**: 821-828

S- Editor Liu Y **L- Editor** Kumar M **E- Editor** Bi L

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5577-5578
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Unusual site of recurrent musculoskeletal hydatid cyst: Case report and brief review of the literature

Panagiotis G Drimousis, Konstantinos M Stamou, Athanasios Koutras, Dimitrios Konstantinos Tsekouras, George Zografos

Panagiotis G Drimousis, Konstantinos M Stamou, Athanasios Koutras, Dimitrios Konstantinos Tsekouras, George Zografos, 1st Department of Propeudeutic Surgery, Hippocrateion Hospital, Athens Medical School, Athens 11527, Greece
Correspondence to: Dr. Panagiotis G Drimousis, 8 Ar. Oikonomou St., Patissia, Athens 14045, Greece. pdrimousis@hippocratio.gr
Telephone: +30-210-8547691
Received: 2006-04-09 Accepted: 2006-05-22

Abstract

A case of a large multiplex recurrent hydatid cyst involving the left gluteal muscle and the left iliopsoas, accompanied with degeneration of the musculature of the left upper leg is presented along with a review of the relevant literature. Very few such cases have been reported worldwide. The presented case is also distinguished by the involvement of muscles of distant anatomic areas.

Key words: Echinococcosis; Hydatid disease; Musculoskeletal hydatid

Drimousis PG, Stamou KM, Koutras A, Tsekouras DK, Zografos G. Unusual site of recurrent musculoskeletal hydatid cyst: Case report and brief review of the literature. *World J Gastroenterol* 2006; 12(34): 5577-5578

http://www.wjgnet.com/1007-9327/12/5577.asp

INTRODUCTION

Echinococcosis is a zoonotic infection caused by tapeworms of the genus *Echinococcus* which inhabits in the small intestine of carnivores. The adult worms produce eggs that are released with the feces and spread in various ways, such as through the wind, water or flies[2]. After ingestion by the host, the embryos migrate through the intestinal wall and are either arrested in the capillary bed of the liver developing into liver cysts, or manage to penetrate into systemic circulation thus ending up in remote organs. The lung, the brain, and the muscles or bones are the more frequently involved distant organs. Due to their physiologic role as capillary filters and their vast capillary volume, the liver and lung are most often affected. Other manifestations are found in 15% of the patients, with the skeletal system making up for 1%-4% of all cases[7]. Voluntary muscles are a very rare site of infection, counting for less than 1% of total[3].

In this report, we present a case of a large multiplex recurrent hydatid cyst involving the left gluteal muscle and the left iliopsoas, accompanied with degeneration of the musculature of the left upper leg. To our knowledge, less than 50 such cases have been reported in the literature worldwide. The presented case is also distinguished by the involvement of muscles of distant anatomic areas, while no liver, lung or bone lesions were identified.

CASE REPORT

A 78-year-old Caucasian male was admitted to our clinic, with a swelling of the waist and the left upper leg. The patient had been operated four times over the past 18 years for echinococcosis of the musculature of the left upper leg. During this period, he suffered recurrent swellings of the thigh that were treated as abscesses. His past medical record was otherwise unremarkable.

On physical examination, a mass was located in the left waist and the upper part of the left gluteus muscle. A communicating fistula with a daughter cyst was identified in the median part of the left gluteus muscle. Another communicating fistula extended from the daughter cyst to 5 cm above the knee joint where a third cyst was palpable. The aforementioned findings were also visible on ultrasound (US) and MRI studies. The lesions of the primary and daughter cyst in the waist and the communicating fistula were located superficially at the fascia of the left gluteus muscle (maximum diameter 10 cm).

There were no signs of calcification in any of these cysts. No liver or lung manifestations were present. Anti-echinococcal IgG was positive for infection (title 2,2, positive if > 1.1, ELISA).

The patient was submitted to perform a complete cystopericystectomy of both primary and daughter cysts and radical excision of the fistula. The overlying skin was excised and the surgical wound was primarily closed. The lesions were easily dissected from the underlying fascia that did not need removal. The cysts contained purulent material. The wound was drained for 24 h. The surgical

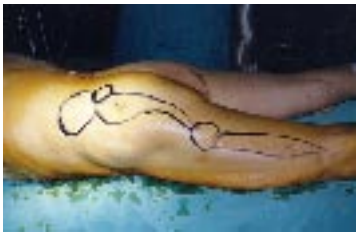


Figure 1 Preoperative appearance of the lesion, where primary cyst, communicating fistulas and daughter cysts are discernible.

specimen was submitted for histopathological examination which confirmed the diagnosis. The postoperative course was uneventful and the patient was discharged on the 6th postoperative day. He has been followed up for 16 mo and no recurrent cyst was evident on CT and MRI scan.

DISCUSSION

Soft tissue hydatid disease is rare even in endemic areas, such as the Mediterranean. Intramuscular lesions in the absence of liver, lung or bone manifestations are most uncommon[1,4]. In our case, we encountered an extended soft tissue disease with no signs of systemic infection but a history of multiple recurrences. To our knowledge, very few similar cases have been reported in the literature worldwide (Table 1). Recurrences had developed in the form of abscess-like lesions in multiple sites of the leg which is also uncommon in intramuscular infections. Quality of life of this patient was poor due to constant symptomatic hymenitis, possibly a result of chronic local inflammation. He also experienced mobilization difficulties(Figure 1).

Determining the ideal therapeutic approach for a recurrent musculosceletal hydatid cyst can be quite challenging for the general surgeon. Moreover, the rarity of the disease renders the decision making on the favorable treatment quite difficult. Conservative treatment, complete excision and simple drainage have all been suggested as adequate[10]. Hydatid disease progresses slowly and is rarely life-threatening, especially when located in the soft tissue or muscles, thus supporting a more conservative therapeutic approach. Additionally, co-morbid conditions and advanced age of the patient as well as the surgeon's experience are of great importance for the final decision. However, in case the disease causes profound disabilities or mobilization problems, complete cystopericystectomy should always be considered.

The nature of the lesion should be well documented and evaluated. Radical surgical therapy is especially indicated in cases of unilocular manifestations as only this method offers hope of permanent cure[9]. Therapeutic dilemmas could arise in cases of extended disease with many muscles or muscle layers in different sites of the body which are communicating *via* fistulas. Communication between lesions should always be suspected and revealed, even if primary and daughter cysts are distant. Complete surgical treatment should include the primary lesion, the daughter cysts and the communicating fistulas as a whole specimen.

Hydatid disease may occur anywhere in the musculoskeletal system, from the big toe to the crown of the head[5,6,8]. In endemic areas, echinococcosis should be always suspected and bared in mind in the differential diagnosis of cystic lesions in soft tissue, even if the radiological appearance is not typical. Once the diagnosis is established, the surgeon should consider performing a radical procedure aiming in minimizing the possibility of a recurrence.

Table 1 Reported sites of intramuscular infection and bone, liver or lung involvement[2,3]

Author	*n*	Site of infection	Liver/lung/bone involvement
Merkle *et al*[2]	8	Iliopsoas, left adductor musculature, left femur, left medial gluteal muscle, musculature of right upper leg	Yes
Rieber *et al*[8]	1	Paravertebral structures	Yes
Sennaroglu *et al*	1	Infratemporal	Yes
Von Sinner[11]	1	Pelvic	Yes
Torricelli *et al*[12]	14	Bone infection with adjacent soft tissue involvement in 12 cases	
Aydin *et al*[13]	1	Cerebral	Yes
Duncan *et al*[4]	1	Biceps brachii	No
Dahniya *et al*[3]	7	5 bone infections without soft tissue involvement, 2 primary intramuscular (left shoulder, rectus femoris and vastus lateralis)	No

REFERENCES

1 **Alldred AJ**, Nisbet NW. Hydatid disease of bone in Australasia. *J Bone Joint Surg Br* 1964; **46**: 260-267
2 **Bourée** P. Hydatidosis: dynamics of transmission. *World J Surg* 2001; **25**: 4-9
3 **Dahniya MH**, Hanna RM, Ashebu S, Muhtaseb SA, el-Beltagi A, Badr S, el-Saghir E. The imaging appearances of hydatid disease at some unusual sites. *Br J Radiol* 2001; **74**: 283-289
4 **Duncan GJ**, Tooke SM. Echinococcus infestation of the biceps brachii. A case report. *Clin Orthop Relat Res* 1990; 247-250
5 **Guthrie JA**, Lawton JO, Chalmers AG. Case report: The MR appearances of primary intramuscular hydatid disease. *Clin Radiol* 1996; **51**: 377-379
6 **Lewall DB**. Hydatid disease: biology, pathology, imaging and classification. *Clin Radiol* 1998; **53**: 863-874
7 **Merkle EM**, Schulte M, Vogel J, Tomczak R, Rieber A, Kern P, Goerich J, Brambs HJ, Sokiranski R. Musculoskeletal involvement in cystic echinococcosis: report of eight cases and review of the literature. *AJR Am J Roentgenol* 1997; **168**: 1531-1534
8 **Rieber A**, Brambs HJ, Friedl P. [CT in echinococcosis of the lumbar spine and paravertebral structures]. *Rofo* 1989; **151**: 379-380
9 **Saimot AG**. Medical treatment of liver hydatidosis. *World J Surg* 2001; **25**: 15-20
10 **Sielaff TD**, Taylor B, Langer B. Recurrence of hydatid disease. *World J Surg* 2001; **25**: 83-86
11 **von Sinner WN**. A case of primary osseous pelvic hydatid disease (Echinococcus granulosus). *Rofo 1991*; **155**: 88-90
12 **Torricelli P**, Martinelli C, Biagini R, Ruggieri P, De Cristofaro R. Radiographic and computed tomographic findings in hydatid disease of bone. *Skeletal Radiol* 1990; **19**: 435-439
13 **Aydin Y**, Barlas O, Yolaş C, Aydin IH, Ceviz A, Aladağ A, Oren D, Akdemir D. Alveolar hydatid disease of the brain. Report of four cases. *J Neurosurg* 1986; **65**: 115-119

S- Editor Wang J **L- Editor** Kumar M **E- Editor** Bai SH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14: 12(34): 5579-5581
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

An alternative surgical approach to a difficult case of Mirizzi syndrome: A case report and review of the literature

Michael Safioleas, Michael Stamatakos, Constantinos Revenas, Constantinos Chatziconstantinou, Constantinos Safioleas, Alkiviades Kostakis

Michael Safioleas, Michael Stamatakos, Constantinos Safioleas, Alkiviades Kostakis, 2nd Department of Propedeutic Surgery, School of Medicine, Athens University, Laiko Hospital, Greece
Constantinos Revenas, Constantinos Chatziconstantinou, Department of Radiology, Laiko Hospital, Athens, Greece
Correspondence to: Professor Michael Safioleas, MD, PhD,7 Kyprou Ave. Filothei, 15237 Athens, Greece. stamatakosmih@yahoo.gr
Telephone: +30-210-6812188
Received: 2006-05-27 Accepted: 2006-06-15

Safioleas M, Stamatakos M, Revenas C, Chatziconstantinou C, Safioleas C, Kostakis A. An alternative surgical approach to a difficult case of Mirizzi syndrome: A case report and review of the literature. *World J Gastroenterol* 2006; 12(34): 5579-5581

http://www.wjgnet.com/1007-9327/12/5579.asp

Abstract

Mirizzi syndrome (MS) is an uncommon complication of gallstone disease and occurs in approximately 1% of all patients suffering from cholelithiasis. The syndrome is characterized by extrinsic compression of the common hepatic duct frequently resulting in clinical presentation of intermittent or constant jaundice. Most cases are not identified preoperatively. Surgery is the indicated treatment for patients with MS. We report here a 71-year-old male patient referred to the surgical outpatient department for diffuse upper abdominal pain and mild jaundice (bilirubin rate: 4.2 mg/dL). Ultrasound examination revealed a stone in the cystic duct compressing the common hepatic duct. The patient had a history of gastrectomy for gastric ulcer 30 years ago. MRCP revealed a stone impacted in the cystic duct causing obstruction of the common hepatic duct by extrinsic compression. With these findings the preoperative diagnosis was indicative of MS. At laparotomy a moderately shrunken gallbladder was found embedded in adhesions containing a large stone which was palpable in the common bile duct. The anterior wall of the body of the gallbladder was opened by an incision which extended longitudinally along the gallbladder towards the common bile duct. The stone measuring 3.0 cm in diameter, was then removed setting astride a large communication with the common bile duct. A Roux-en-Y cholecysto-choledocho-jejunostomy was performed. The subhepatic region was drained. The patient had an uneventful recovery. He was discharged eleven days after operation and remained well after a 30-mo follow-up.

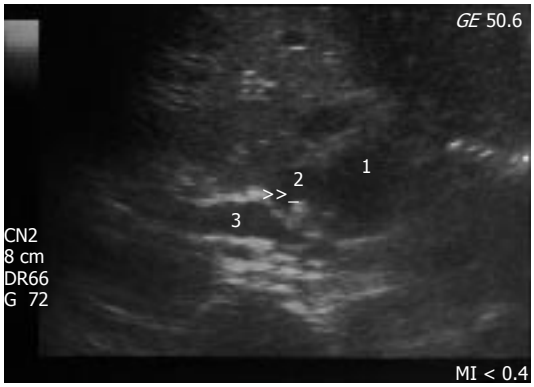
Key words: Benign jaundice; Hepatic duct obstruction; Impacted gallstone; Cholecystobiliary fistula

INTRODUCTION

Mirizzi syndrome (MS) is a rare complication of long-standing cholelithiasis, which results from impaction of a large calculus or multiple small stones in the cystic duct or in the neck of the gallbladder causing extrinsic narrowing of the common hepatic duct. This condition may result in the clinical presentation of intermittent or constant jaundice. MS occurs in approximately 1% of all patients with cholelithiasis. Although modern imaging techniques are available, the majority of cases are identified during surgery. During the last two decades, 27 patients suffering from MS have been treated in our department[1,2], we present here a case of a 71-year-old male patient with MS and a literature review.

CASE REPORT

A 71-year-old male patient was referred to the surgical outpatient department for diffuse upper abdominal pain and mild jaundice (bilirubin rate: 4.2 mg/dL). Ultrasound examination revealed a stone in the cystic duct compressing the common hepatic duct (Figure 1). The patient had a history of gastrectomy for gastric ulcer 30 years ago, thus ERCP was not feasible. MRCP revealed a stone impacted in the cystic duct causing obstruction of the common hepatic duct by extrinsic compression. With these findings the preoperative diagnosis was indicative of MS. At laparotomy a moderately shrunken gallbladder was found embedded in adhesions containing a large stone which was palpable in the common bile duct (Figure 2A). It was obvious that the local operative circumstances required great surgical care. Therefore the anterior wall of the body of the gallbladder was opened by an incision which extended longitudinally along the gallbladder towards the common bile duct. The cystic duct could not be identified. The stone measuring 3.0 cm in diameter was then removed setting astride a large



1 Gall bladder
2 Cystic duct
3 Common hepatic duct
>> Stone

Figure 1 Ultrasound showing a stone compressing the common hepatic duct.

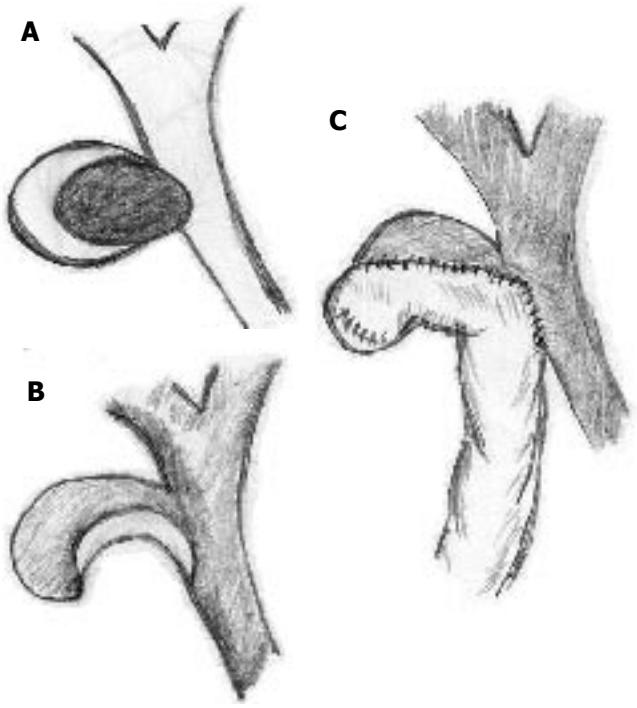


Figure 2 Schematic representation of the described technique during laparotomy (**A**, **B**) and Roux-en-Y cholecysto-choledocho-jejunostomy (**C**).

communication with the common bile duct (Figure 2B). Based on this finding and because the risk of stricture at the site of fistulae was significant, we decided to bypass the cholecystocholedochal fistulus defect rather than to close it directly or by using a gallbladder flap for closing the opening of the common bile duct around a T-tube. A Roux-en-Y cholecysto-choledocho-jejunostomy was performed (Figure 2C). The subhepatic region was drained. The patient had an uneventful recovery. He was discharged on the 11th postoperative day and remained well after a 30-mo follow-up.

DISCUSSION

MS was first described in 1948 as obstructive jaundice due to a gallstone impacted in the cystic duct or Hartmann's pouch compressing the common hepatic duct[3].

McSherry *et al*[4] in 1982 suggested a subclassification of MS into two types. The first type concerns the external compression of the common hepatic duct by a calculus in the cystic duct or Hartmann's pouch, whereas in the second type the stone has entered partly or completely into the common bile duct, resulting in a cholecysto-choledochal fistula. Furthermore, in 1989 a new classification of patients with MS and cholecystobiliary fistulae was presented by Csendes *et al*[5], which includes four types: type I lesion includes those with external compression of the common bile duct; type Ⅱ lesion is a cholecystobiliary fistula present with erosion of less than one third of the circumference of the bile duct; type Ⅲ lesion is a fistula involving up to two-thirds of the duct circumference; type Ⅳ lesion is a complete destruction of the bile duct.

MS and cholecystobiliary fistulae therefore appear to be different, evolving stages of the same pathological condition, thus it is reasonable that Lubbers[6] proposes that the term MS can now be abandoned, since it is only the first stage of a more complex process.

Gallstone erosion into the common duct is nevertheless a rare complication of cholelithiasis with an incidence rate ranging from 0.7% to 1.4% of all patients undergoing cholecystectomy[7].

The clinical diagnosis of MS is difficult, since there are no pathognomonic patterns of presentation. Ultrasound is diagnostically the best screening method, with ERCP and/or MRCP to confirm the diagnosis. MRCP can be as good as ERCP in the diagnosis and its ability to delineate details of biliary structures, but its disadvantage compared to ERCP is its inability to confirm the presence of fistulae and does not afford therapeutic stenting. On the other hand, T_2 weighted sections can differentiate a neoplastic mass from an inflammatory one, that cannot be detected by ultrasonogram or CT scan[8]. Finally intraductal ultrasound, as an adjunct to ERCP, can also be of help[9]. Despite of all these modern diagnostic tools, the problem may become apparent only during surgery.

Surgical treatment for type I MS is partial cholecystectomy leaving the neck of the gallbladder in place[10]. In some cases, open or laparoscopic total cholecystectomy may be performed[11]. However some authors consider this a contraindication for laparoscopic cholecystectomy[12-14].

Surgical treatment of type Ⅱ MS is less clearly defined. Corlette and Bismuth[15] have recommended partial cholecystectomy, oversuturing of the gallbladder cuff and insertion of a T-tube through the fistula as an adequate treatment for type Ⅱ MS.

Choledochoplasty is an acceptable therapeutic approach but the amount of gallbladder tissue employed for this has not yet been standardized[16].

Furthermore, cholecystoduodenostomy has been described[17] and hepaticojejunectomy[18] can also be used if complete destruction of the common hepatic duct occurs.

Reconstruction of the extrahepatic biliary tree in case of MS type Ⅱ with bypass of the lesion using a Roux-

en-Y cholecysto-choledochal-jejunostomy as in our case can be carried out. To our knowledge, this is the first case described in the literature.

In conclusion, since the preoperative diagnosis of MS cannot be achieved, an awarded suspicion is necessary to avoid a lesion of the biliary tree if firm adherence around Carlot's triangle is found. The success of treatment is related to a precocious recognition of the condition even at the time of surgery when the individual characteristics of each case are considered[18].

REFERENCES

1 **Safioleas M**, Yiagos E, Doka P, Koskinas A, Stefanou J, Skalkeas Gr. Mirizzi Syndrome. *Medical Annals* 1990; **13**: 863-866
2 **Safioleas M**, Evagelidakis E, Xypolitas N, Liossi A. Mirizzi Syndrome. *Medical Annals* 1999; **12**: 545-547
3 **Mirizzi PL**. Syndrome del conducto hepatico. *Journal International de Chirurgie* 1948; **8**: 731-737
4 **McSherry CK**, Ferstenberg H, Virshup M. The Mirizzi syndrome: suggested classification and surgical therapy. *Surg Gastroenterol* 1982; **1**: 219-225
5 **Csendes A**, Díaz JC, Burdiles P, Maluenda F, Nava O. Mirizzi syndrome and cholecystobiliary fistula: a unifying classification. *Br J Surg* 1989; **76**: 1139-1143
6 **Lubbers EJ**. Mirizzi syndrome. *World J Surg* 1983; **7**: 780-785
7 **Pemberton M**, Wells AD. The Mirizzi syndrome. *Postgrad Med J* 1997; **73**: 487-490
8 **Choi BW**, Kim MJ, Chung JJ, Chung JB, Yoo HS, Lee JT. Radiologic findings of Mirizzi syndrome with emphasis on MRI. *Yonsei Med J* 2000; **41**: 144-146
9 **Phatak N**, Kochman ML. Biliary endoscopy. *Curr Opin Gastroenterol* 2004; **20**: 281-287
10 **Abou-Saif A**, Al-Kawas FH. Complications of gallstone disease: Mirizzi syndrome, cholecystocholedochal fistula, and gallstone ileus. *Am J Gastroenterol* 2002; **97**: 249-254
11 **Yeh CN**, Jan YY, Chen MF. Laparoscopic treatment for Mirizzi syndrome. *Surg Endosc* 2003; **17**: 1573-1578
12 **Posta CG**. Unexpected Mirizzi anatomy: a major hazard to the common bile duct during laparoscopic cholecystectomy. *Surg Laparosc Endosc* 1995; **5**: 412-414
13 **Bagia JS**, North L, Hunt DR. Mirizzi syndrome: an extra hazard for laparoscopic surgery. *ANZ J Surg* 2001; **71**: 394-397
14 **Tan KY**, Chng HC, Chen CY, Tan SM, Poh BK, Hoe MN. Mirizzi syndrome: noteworthy aspects of a retrospective study in one centre. *ANZ J Surg* 2004; **74**: 833-837
15 **Corlette MB**, Bismuth H. Biliobiliary fistula. A trap in the surgery of cholelithiasis. *Arch Surg* 1975; **110**: 377-383
16 **Shah OJ**, Dar MA, Wani MA, Wani NA. Management of Mirizzi syndrome: a new surgical approach. *ANZ J Surg* 2001; **71**: 423-427
17 **Baer HU**, Matthews JB, Schweizer WP, Gertsch P, Blumgart LH. Management of the Mirizzi syndrome and the surgical implications of cholecystcholedochal fistula. *Br J Surg* 1990; **77**: 743-745
18 **Johnson LW**, Sehon JK, Lee WC, Zibari GB, McDonald JC. Mirizzi's syndrome: experience from a multi-institutional review. *Am Surg* 2001; **67**: 11-14

S- Editor Wang J **L- Editor** Wang XL **E- Editor** Bi L

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5582-5586
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Portal venous gas and thrombosis in a Chinese patient with fulminant Crohn's colitis: A case report with literature review

Simon Siu-Man Ng, Raymond Ying-Chang Yiu, Janet Fung-Yee Lee, Jimmy Chak-Man Li, Ka-Lau Leung

Simon Siu-Man Ng, Raymond Ying-Chang Yiu, Janet Fung-Yee Lee, Jimmy Chak-Man Li, Ka-Lau Leung, Department of Surgery, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong SAR, China
Correspondence to: Dr. Simon Siu-Man Ng, Department of Surgery, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong SAR, China. simonng@surgery.cuhk.edu.hk
Telephone: +852-26321495 Fax: +852-26377974
Received: 2006-04-24 Accepted: 2006-05-22

Key words: Portal venous gas; Portal vein thrombosis; Pylephlebitis; Crohn's disease; Chinese

Ng SSM, Yiu RYC, Lee JFY, Li JCM, Leung KL. Portal venous gas and thrombosis in a Chinese patient with fulminant Crohn's colitis: A case report with literature review. *World J Gastroenterol* 2006; 12(34): 5582-5586

http://www.wjgnet.com/1007-9327/12/5582.asp

Abstract

Ever since its earliest reports, portal venous gas (PVG) has been associated with numerous intraabdominal catastrophes and has served as an indication for urgent surgical exploration. It is traditionally regarded to be an ominous finding of impending death, with highest mortality reported in patients with underlying bowel ischemia. Today, computed tomography has demonstrated a wider range of clinical conditions associated with PVG, some of which are 'benign' and do not necessarily require surgery, unless when there are signs of intraabdominal catastrophe or systemic toxicity. One of these 'benign' conditions is Crohn's disease. The present report describes a 19-year-old Chinese boy with Crohn's pancolitis who presented with septic shock associated with PVG and portal vein thrombosis, and was successfully managed surgically. To our knowledge, this is the first report of PVG and portal vein thrombosis associated with Crohn's disease in a Chinese patient. In addition, we have also reviewed the reports of another 18 Crohn's patients with PVG previously described in the English literature. Specific predisposing factors for PVG were identified in 8 patients, including barium enema, colonoscopy, blunt abdominal trauma, and enterovenous fistula. The patients who developed PVG following barium enema and blunt trauma were all asymptomatic and no specific treatment was necessary. Eleven patients (58%) who presented with signs of intraabdominal catastrophe or systemic toxicity required either immediate or eventual surgery. The overall mortality rate among the 19 patients was only 11%. The present literature review has shown that the finding of PVG associated with Crohn's disease does not always mandate surgical intervention. It is the clinical features and the related complications that ultimately determine the treatment approaches. The overall outcome of PVG associated with Crohn's disease has been favourable.

INTRODUCTION

Portal venous gas (PVG) is a rare radiological finding that occurs when intraluminal gas from the gastrointestinal tract or gas-forming bacteria enters the portal venous circulation[1]. Factors predisposing to PVG include bowel mucosal injury, bowel distension, and sepsis[2]. Ever since its earliest reports[3,4], PVG has been associated with numerous intraabdominal catastrophes and has served as an indication for urgent surgical exploration. It is traditionally regarded to be an ominous finding of impending death, with highest mortality reported in patients with underlying bowel ischemia[2]. However, it is becoming apparent that there are conditions in which the finding of PVG is relatively 'benign' and does not always indicate surgery, and these conditions include digestive tract dilatation, ulcerative colitis, Crohn's disease, and complications of iatrogenic and endoscopic procedures[5]. Remarkably, there are reports of PVG occurring in patients with uncomplicated Crohn's disease after blunt trauma[6,7] or colonic diagnostic procedures[8,9], which resolves spontaneously without treatment. On the other hand, when PVG occurs in Crohn's patients with signs of intraabdominal catastrophe or systemic toxicity, urgent surgery is warranted, and the final outcome has mostly been favourable[10-18]. The present report describes a 19-year-old Chinese boy with Crohn's pancolitis who presented with septic shock associated with PVG and portal vein thrombosis, and was successfully managed surgically. To our knowledge, this is the first report of PVG and portal vein thrombosis associated with Crohn's disease in a Chinese patient. The literature of PVG associated with Crohn's disease is also reviewed, with special emphasis on the clinical features, the various predisposing factors, and the treatment approaches.

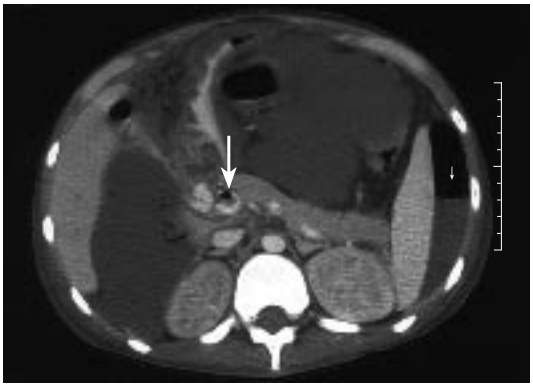


Figure 1 Computed tomography of the abdomen showing evidence of portal venous gas (long arrow), portal vein thrombosis, gross ascites, and pneumoperitoneum (short arrow).

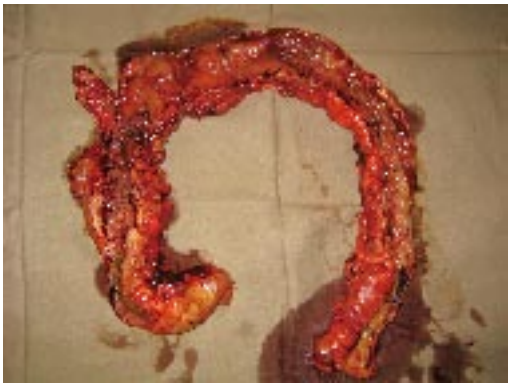


Figure 2 Photograph of the total colectomy specimen showing the classical features of Crohn's colitis: cobblestone mucosa with skipped lesions.

CASE REPORT

A 19-year-old Chinese boy presented to the Accident and Emergency Department of our hospital with fever, hypotension, and abdominal distension. He had been diagnosed with Crohn's pancolitis a year ago and had been treated with mesalazine, but the drug compliance was poor. One month prior to this hospitalization, he had been admitted to another hospital because of fever and bloody diarrhoea. He was treated as a flare-up of Crohn's disease with intravenous steroid and antibiotics. However, his condition remained static despite a week of medical treatment, and he insisted to be discharged against medical advice. He consulted a Chinese herbalist and consumed some Chinese herbal medicines, but his condition continued to deteriorate. He became so ill that his parents finally brought him to our hospital to seek for further medical treatment.

On arrival, his temperature was 38℃, blood pressure 86/40 mmHg and heart rate 153 beats/min respectively. The abdomen was grossly distended and tense, although no frank peritoneal sign could be elicited. Initial blood tests showed leukocytosis, coagulopathy, and metabolic acidosis. He was immediately admitted to the Intensive Care Unit (ICU); aggressive fluid resuscitation and antibiotics were given. After initial stabilization, an urgent computed tomography (CT) of the abdomen and pelvis was performed, which showed evidence of portal venous gas, portal vein thrombosis, gross ascites, and pneumoperitoneum (Figure 1). The small bowel appeared thickened and inflamed, but the large bowels were not clearly demonstrated. The diagnosis was compatible with Crohn's disease with bowel perforation and septic shock, and hence an emergency laparotomy was arranged.

Intraoperatively, generalized peritonitis with 2-3 L of faeculent fluid and pus was noted throughout the peritoneal cavity. The small and large bowels were densely matted together by inflammatory adhesions. The large bowel appeared shrunken and chronically diseased, but no perforated site was identified. No obvious stricture or perforation was noted along the small bowel. The adhesions between the bowels were removed, and this already resulted in a blood loss of 9 L because of severe coagulopathy. Damage control surgery with packing of all the raw areas was done and the patient was sent back to the ICU for

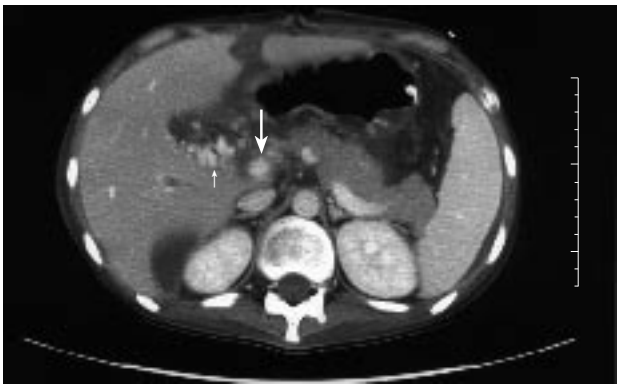


Figure 3 Computed tomography of the abdomen showing evidence of partial recanalization of the portal vein (long arrow) with increasing surrounding collaterals (short arrow).

further stabilization. NovoSeven (recombinant coagulation factor VIIa) and blood products were given to correct the coagulopathy. A second-look laparotomy performed 24 h later showed no more active bleeding from the raw areas. No obvious perforated site was identified along the gastrointestinal tract. The small bowel appeared edematous but viable, while the large bowel appeared shrunken and unhealthy. Thus a total colectomy was performed to remove the diseased large bowel (Figure 2). The abdomen was temporarily closed using a sterile plastic bag to avoid abdominal compartment syndrome. The abdomen was subsequently closed at a 3rd laparotomy 48 h later and an end ileostomy was fashioned. Pathological examination of the resected colon showed severe Crohn's colitis with multiple ulcerations and deep fissures but without perforation.

The postoperative course was very stormy and the patient had prolonged stay in the ICU. Peritoneal swab cultures and blood cultures grew *Enterococcus* and Methicillin-resistant *Staphylococcus aureus* and so vancomycin was added to the treatment regimen. For the portal vein thrombosis, anticoagulation therapy was not started initially because of coagulopathy. Nevertheless, partial recanalization of the portal vein with increasing collaterals was evident on follow-up CT a few weeks later (Figure 3). One month after the 3rd surgery, he developed an episode of massive intraabdominal haemorrhage secondary to erosion of a

Table 1 Clinical features, predisposing factors, treatment, and outcome of 19 Crohn's patients with PVG reported in the English literature

Author (yr)	Sex/age	Clinical features	Predisposing factors	Diagnostic modality	Treatment	Operative findings	Outcome
Reiner *et al* (1978)[10]	F/34	Fever and abdominal pain	Enterovenous fistula	Plain X-ray	Antibiotics and surgery	Ileitis	Survived
Sadhu *et al* (1979)[8]	F/64	No symptom or morbidity	Barium enema	Plain X-ray	No treatment	/	Survived
Gosink (1981)[11]	M/45	Fever and abdominal pain	/	US	Surgery	Inflamed colon	Survived
Pappas *et al* (1984)[9]	M/36	No symptom or morbidity	Sigmoidoscopy, barium enema	Plain X-ray	No treatment		Survived
Huycke *et al* (1985)[12]	M/22	Resolution of toxic megacolon; developed abdominal pain and free peritoneal air after colonoscopy	Colonoscopy	Plain X-ray	Antibiotics and surgery	Ileitis and colitis, no perforation	Survived
Katz *et al* (1986)[21]	M/14	No symptom or morbidity	Barium enema	Plain X-ray	Antibiotics		Survived
Ajzen *et al* (1988)[13]	M/64	Severe epigastric pain	Enterovenous fistula	US, CT	Surgery	Ileitis	Died of sepsis and liver failure 1 mo later
Venugopal *et al* (1990)[14]	F/27	Fever	/	CT	Surgery	Ileitis, no perforated or ischaemic bowel	Survived
Kirsch *et al* (1990)[22]	F/26	Epigastric pain and chills	/	Plain X-ray, CT	Antibiotics	/	Survived
Delamarre *et al* (1991)[23]	M/70	Fever	/	CT	Antibiotics	/	Survived
al-Jahdali *et al* (1994)[15]	F/40	Fever	/	CT	Antibiotics (Surgery 2 wk later)	Ileitis	Survived
Hong *et al* (1997)[16]	M/58	Fever, status post-low anterior resection and small bowel resection	/	CT	Antibiotics	/	Survived
Hong *et al* (1997)[16]	F/71	Abdominal pain (Developed free peritoneal air 2 wk later)	/	CT	Antibiotics (Surgery 2 wk later)	Ischaemic and perforated small bowel	Died of disseminated cytomegalovirus infection
Hong *et al* (1997)[16]	M/24	Fever (Developed abdominal pain 4 wk later)	/	CT	Antibiotics (Surgery 4 wk later)	Ileitis	Survived
Brandon *et al* (2000)[17]	F/59	Fever and abdominal pain	/	CT	Surgery	Ileitis and colitis	Survived
Nesher *et al* (2002)[6]		No symptom or morbidity	Blunt trauma	CT	No treatment	/	Survived
Paran *et al* (2003)[7]	F/25	No symptom or morbidity	Blunt trauma	CT	No treatment	/	Survived
Thethy *et al* (2005)[18]	F/58	Fever	/	CT	Surgery	Sigmoid inflammatory mass	Survived
Present case	M/19	Septic shock with free peritoneal air	/	CT	Surgery	Colitis, no perforation	Survived

US: Ultrasonography; CT: Computed tomography.

mesenteric vessel by an infected collection and required a 4th laparotomy for haemostasis. Fortunately, he recovered quite uneventfully thereafter and was subsequently discharged home 2 mo after the first surgery.

The patient was put on maintenance mesalazine and azathioprine and remained well and asymptomatic 14 mo after the surgery. A CT enteroclysis was performed later and showed no evidence of small bowel involvement.

DISCUSSION

Portal venous gas was first described by Wolfe and Evans in 1955 in 6 neonates with fatal necrotizing enterocolitis[3]. In 1960, Susman and Senturia reported the similar finding in an adult, critically ill with small bowel infarction[4]. Since then, PVG has been reported with increasing frequency in the literature. One of the first reviews by Liebman *et al* evaluated 64 patients with PVG on plain X-ray reported in the literature before 1977[2]. According to that review, although PVG was observed in association with various clinical conditions, it was mostly (72%) found among patients seriously ill with necrotic bowel. The overall mortality was 75%, and this led the authors to recommend urgent surgical exploration for PVG except for patients with stable

ulcerative colitis who had undergone barium enemas, because 5 such patients survived with conservative treatment. Over the last 2 to 3 decades, advances in diagnostic radiology, including the development of ultrasonography and CT, have increased the sensitivity of imaging PVG. Brandon *et al* suggested that early recognition using CT, with appropriate surgical intervention, improves the chance for patient survival when PVG is identified[17]. Moreover, PVG on CT has been found to be associated with a wider range of clinical conditions, some of which are 'benign' and do not necessarily require surgical intervention, especially when there are no signs of intraabdominal catastrophe or systemic toxicity[19]. A recent study of 17 patients with PVG detected by CT has actually reported a mortality rate as low as 29%[20]. Today, PVG is recognized as a mere diagnostic clue in patients with suspected acute abdominal pathology and is not itself a predictor of mortality. The more relevant prognosticator is the clinical condition in which PVG occurs.

One of the relatively 'benign' conditions that are associated with PVG is Crohn's disease. Including the present case, 19 cases of PVG associated with Crohn's disease were reported in the English literature (Table 1)[6-18,21-23]. In 4 of these patients, PVG was iatrogenic in origin, resulting from barium enema[8,9,21] or colonoscopy[12]. Two patients developed PVG after blunt abdominal trauma[6,7]. It has been postulated that elevated intraluminal pressure (during colonic diagnostic procedures) or intraperitoneal pressure (associated with blunt trauma) can permit bowel gas or gas-forming bacteria to gain access to the portal venous circulation through microscopic mucosal injury. Another factor predisposing to PVG is the development of enterovenous fistula between the bowel lumen and the mesenteric venous system, which is an extremely rare complication reported in 2 patients with Crohn's disease[10,13]. In the remaining 11 patients, no specific predisposing factors could be identified. PVG in these patients is thought to be the result of mucosal injury and sepsis[2] associated with bowel inflammation and portal pyaemia. The occurrence of PVG does not seem to be associated with the anatomical location of Crohn's disease involvement. Both ileal and colonic diseases can develop PVG.

The finding of PVG associated with Crohn's disease does not always mandate surgical intervention. It is the clinical features and the related complications that ultimately determine the treatment approaches. The presentation of PVG following blunt trauma[6,7] and barium enema[8,9,21] is remarkably innocuous; all the 5 reported patients were asymptomatic and no specific treatment was necessary (except in 1 patient who was given 'prophylactic antibiotics' for 48 h[21]). Intravenous antibiotics were administered to patients who developed fever, and 3 of them had complete resolution of symptoms with this simple medical treatment[16,22,23]. Eleven patients (58%) who presented with signs of intraabdominal catastrophe or systemic toxicity required either immediate or eventual surgery. All these patients underwent resection of the inflamed small and large bowels. The overall mortality rate among the 19 patients was only 11%; a patient with enterovenous fistula died of sepsis and liver failure 1 mo after surgery[13], while another patient who underwent surgery for ischemic and perforated small bowel finally died of disseminated cytomegalovirus infection[16].

The combination of extensive pylephlebitis (or septic thrombosis of the portal vein) and PVG that occurs in our case is an extremely rare complication among patients with Crohn's disease; only 1 similar case was reported in the German literature[24]. Severe active Crohn's disease and sepsis are the 2 predisposing factors[25]. Although PVG itself is not a predictor of mortality, the finding of PVG combined with pylephlebitis is generally regarded as an ominous prognostic sign[26]. Nevertheless, as illustrated in our case, favourable clinical outcome in these patients can still be achieved with early CT diagnosis, maximal organ support in the ICU, aggressive medical treatment, and prompt surgical intervention.

In conclusion, the finding of PVG associated with Crohn's disease does not always mandate surgical intervention. It is the clinical features and the related complications that ultimately determine the treatment approaches. The overall outcome of PVG associated with Crohn's disease has been favourable.

REFERENCES

1 **Hou SK**, Chern CH, How CK, Chen JD, Wang LM, Lee CH. Hepatic portal venous gas: clinical significance of computed tomography findings. *Am J Emerg Med* 2004; **22**: 214-218
2 **Liebman PR**, Patten MT, Manny J, Benfield JR, Hechtman HB. Hepatic-portal venous gas in adults: etiology, pathophysiology and clinical significance. *Ann Surg* 1978; **187**: 281-287
3 **Wolfe JN**, Evans WA. Gas in the portal veins of the liver in infants; a roentgenographic demonstration with postmortem anatomical correlation. *Am J Roentgenol Radium Ther Nucl Med* 1955; **74**: 486-488
4 **Susman N**, Senturia HR. Gas embolization of the portal venous system. *Am J Roentgenol Radium Ther Nucl Med* 1960; **83**: 847-850
5 **Kinoshita H**, Shinozaki M, Tanimura H, Umemoto Y, Sakaguchi S, Takifuji K, Kawasaki S, Hayashi H, Yamaue H. Clinical features and management of hepatic portal venous gas: four case reports and cumulative review of the literature. *Arch Surg* 2001; **136**: 1410-1414
6 **Nesher E**, Aizner A, Kashtan H, Kaplan O, Kluger Y, Greenberg R. Portal vein air embolization after blunt abdominal trauma: a case report and review of the literature. *Eur J Emerg Med* 2002; **9**: 163-165
7 **Paran H**, Epstein T, Gutman M, Shapiro Feinberg M, Zissin R. Mesenteric and portal vein gas: computerized tomography findings and clinical significance. *Dig Surg* 2003; **20**: 127-132
8 **Sadhu VK**, Brennan RE, Madan V. Portal vein gas following air-contrast barium enema in granulomatous colitis: report of a case. *Gastrointest Radiol* 1979; **4**: 163-164
9 **Pappas D**, Romeu J, Tarkin N, Dave PB, Messer J. Portal vein gas in a patient with Crohn's colitis. *Am J Gastroenterol* 1984; **79**: 728-730
10 **Reiner L**, Freed A, Bloom A. Enterovenous fistulization in Crohn's disease. *JAMA* 1978; **239**: 130-132
11 **Gosink BB**. Intrahepatic gas: differential diagnosis. *AJR Am J Roentgenol* 1981; **137**: 763-767
12 **Huycke A**, Moeller DD. Hepatic portal venous gas after colonoscopy in granulomatous colitis. *Am J Gastroenterol* 1985; **80**: 637-638
13 **Ajzen SA**, Gibney RG, Cooperberg PL, Scudamore CH, Miller RR. Enterovenous fistula: unusual complication of Crohn disease. *Radiology* 1988; **166**: 745-746
14 **Venugopal C**, Colley DP. Portal venous gas in a patient with Crohn's disease. *J Clin Gastroenterol* 1990; **12**: 595-597
15 **al-Jahdali H**, Pon C, Thompson WG, Matzinger FR. Non-fatal

portal pyaemia complicating Crohn's disease of the terminal ileum. *Gut* 1994; **35**: 560-561
16 **Hong JJ**, Gadaleta D, Rossi P, Esquivel J, Davis JM. Portal vein gas, a changing clinical entity. Report of 7 patients and review of the literature. *Arch Surg* 1997; **132**: 1071-1075
17 **Brandon T**, Bogard B, ReMine S, Urban L. Early recognition of hepatic portal vein gas on CT with appropriate surgical intervention improves patient survival. *Curr Surg* 2000; **57**: 452-455
18 **Thethy SS,** Potter MA. An unusual complication of Crohn's colitis. *Gut* 2005; **54**: 768, 796
19 **Iannitti DA**, Gregg SC, Mayo-Smith WW, Tomolonis RJ, Cioffi WG, Pricolo VE. Portal venous gas detected by computed tomography: is surgery imperative? *Dig Surg* 2003; **20**: 306-315
20 **Faberman RS**, Mayo-Smith WW. Outcome of 17 patients with portal venous gas detected by CT. *AJR Am J Roentgenol* 1997; **169**: 1535-1538
21 **Katz BH**, Schwartz SS, Vender RJ. Portal venous gas following a barium enema in a patient with Crohn's colitis. A benign finding. *Dis Colon Rectum* 1986; **29**: 49-51
22 **Kirsch M,** Bozdech J, Gardner DA. Hepatic portal venous gas: an unusual presentation of Crohn's disease. *Am J Gastroenterol* 1990; **85**: 1521-1523
23 **Delamarre J**, Capron JP, Dupas JL, Deschepper B, Jouet-Gondry C, Rudelli A. Spontaneous portal venous gas in a patient with Crohn's ileocolitis. *Gastrointest Radiol* 1991; **16**: 38-40
24 **Kluge S**, Hahn KE, Lund CH, Gocht A, Kreymann G. [Pylephlebitis with air in the portal vein system. An unusual focus in a patient with sepsis]. *Dtsch Med Wochenschr* 2003; **128**: 1391-1394
25 **Crowe A**, Taffinder N, Layer GT, Irvine A, Nicholls RJ. Portal vein thrombosis in a complicated case of Crohn's disease. *Postgrad Med J* 1992; **68:** 291-293
26 **Häner C**, Inderbitzi R, Badulescu M, Teuscher J. [Septic pylephlebitis with detection of gas in the portal vein: a rare complication of sigmoid diverticulitis]. *Schweiz Med Wochenschr* 1998; **128**: 1339-1344

S- Editor Pan BR **L- Editor** Zhu LH **E- Editor** Bai SH

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5587
World Journal of Gastroenterology ISSN 1007-9327

Is there an alternative therapy to cyanoacrylate injection for safe and effective obliteration of bleeding gastric varices?

Hiroo Imazu, Kuniyuki Kojima, Masahiko Katsumura, Salem Omar

Hiroo Imazu, Department of Endoscopy, Jikei University School of Medicine, Tokyo, Japan
Kuniyuki Kojima, Masahiko Katsumura, Third Department of Internal Medicine, Nara Medical University, Nara, Japan
Salem Omar, Division of Gastroenterology, Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
Correspondence to: Dr. Hiroo Imazu, Department of Endoscopy, The Jikei University School of Medicine, 3-25-8 Nishi-shinbashi, Minato-ku, Tokyo 105-8461, Japan. himazu21@aol.com
Telephone: +81-3-34331111 Fax: +81-3-34594524
Received: 2006-03-26 Accepted: 2006-04-24

Imazu H, Kojima K, Katsumura M, Omar S. Is there an alternative therapy to cyanoacrylate injection for safe and effective obliteration of bleeding gastric varices? *World J Gastroenterol* 2006; 12(34): 5587

http://www.wjgnet.com/1007-9327/12/5587.asp

TO THE EDITOR

We read with interest the article entitled "Bleeding gastric varices: Results of endoscopic injection with cyanoacrylate at King Chulalongkorn Memorial Hospital" by Noophun *et al*[1]. They performed n-butyl-2-cyanoacrylate (CA) injection therapy for bleeding gastric varices in twenty-four patients, and hemostasis was achieved in seventeen (71%) patients. They concluded that CA injection therapy was effective and safe for bleeding gastric varices. However, we disagreed with the author's conclusion. Their hemostasis rate was relatively low, and two of the 24 patients developed serious complications as a result of glue embolism. Although CA injection therapy has been accepted as the first line treatment especially in Europe and Asia, hemostasis and obliteration of gastric varices are still a therapeutic challenge and many serious complications as a result of CA injection have been reported[2]. In addition, there is no reimbursement from the insurance companies in Japan if CA is used for obliterating varices and therefore this therapeutic option is not widely practised there. Although conventional endoscopic injection sclerotherapy using ethanolamine oleate has been reported to be ineffective for bleeding gastric varices[3], we conducted endoscopic injection sclerotherapy combined with a vasoactive drug for bleeding gastric fundal varices. We have reported a series of thirty patients with bleeding gastric fundal varices treated with endoscopic injection sclerotherapy using 5% ethanolamine oleate plus infusion of vasopressin[4]. With our method, continuous injection of 5% ethanolamine oleate mixed with contrast medium through a double lumen catheter was performed under fluoroscopic guidance until it filled the varices and their feeder veins, and thrombin glue was sprayed at the puncture site during withdrawal of the injector needle to prevent bleeding. We have achieved a high rate of hemostasis (93%) with a relatively low rebleeding rate (19%) over follow-up period of up to 5 years without any serious complications[4]. We would like to recommend our sclerotherapy under fluoroscopic guidance method combined with medical therapy as an alternative to CA injection therapy for bleeding gastric varices, although randomized trials with a larger number of patients are warranted. If CA is to be used, aliquots of 1.0 mL CA per injection must be strictly enforced to prevent any embolic complications. Injections can be repeated if bleeding continues, as long as only 1.0 mL CA is injected each time[5]. Fluoroscopic guidance might also be useful in minimizing this complication[6].

REFERENCES

1 **Noophun P**, Kongkam P, Gonlachanvit S, Rerknimitr R. Bleeding gastric varices: results of endoscopic injection with cyanoacrylate at King Chulalongkorn Memorial Hospital. *World J Gastroenterol* 2005; **11**: 7531-7535
2 **Imazu H**, Seewald S, Omar S, Zhong Y, Soehendra N. Endoscopic treatment for portal hypertension: what's new in the last 12 months? *Endoscopy* 2005; **37**: 116-121
3 **Oho K**, Iwao T, Sumino M, Toyonaga A, Tanikawa K. Ethanolamine oleate versus butyl cyanoacrylate for bleeding gastric varices: a nonrandomized study. *Endoscopy* 1995; **27**: 349-354
4 **Kojima K**, Imazu H, Matsumura M, Honda Y, Umemoto N, Moriyasu H, Orihashi T, Uejima M, Morioka C, Komeda Y, Uemura M, Yoshiji H, Fukui H. Sclerotherapy for gastric fundal variceal bleeding: is complete obliteration possible without cyanoacrylate? *J Gastroenterol Hepatol* 2005; **20**: 1701-1706
5 **Seewald S**, Sriram PV, Naga M, Fennerty MB, Boyer J, Oberti F, Soehendra N. Cyanoacrylate glue in gastric variceal bleeding. *Endoscopy* 2002; **34**: 926-932
6 **Iwase H**, Maeda O, Shimada M, Tsuzuki T, Peek RM Jr, Nishio Y, Ando T, Ina K, Kusugami K. Endoscopic ablation with cyanoacrylate glue for isolated gastric variceal bleeding. *Gastrointest Endosc* 2001; **53**: 585-592

S- Editor Pan BR **L- Editor** Wang XL **E- Editor** Liu WF

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5588
World Journal of Gastroenterology ISSN 1007-9327

ACKNOWLEDGMENTS

Acknowledgments to Reviewers of *World Journal of Gastroenterology*

Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of *World Journal of Gastroenterology*. The editors and authors of the articles submitted to the journal are grateful to the following reviewers for evaluating the articles (including those were published and those were rejected in this issue) during the last editing period of time.

Juan Gonzalez Abraldes, MD
Hepatic Hemodynamic Laboratory, Liver Unit. Hospital Clinic. University of Barcelona, Hepatic Hemodynamic Lab. Liver Unit, Hospital, Clinic, Villarroel 170, Barcelona 08036, Spain

Taiji Akamatsu, Associate Professor
Department of Endoscopy, Shinshu University Hospital, 3-1-1 Asahi, Matsumoto 390-8621, Japan

Sk Md Fazle Akbar, Dr, Assistant Professor
Third Department of Internal Medicine, Ehime University School of Medicine, Shigenobu-Cho, Ehime 791-0295, Japan

Annibale Bruno Annibale, Professor
Digestive and Liver Disease Unit, University " La Sapienza" II School of Medicine, Via di Grottarossa 1035, Roma 00189, Italy

Takeshi Azuma, Associate Professor
Second Department of Internal Medicine, University of Fukui, Faculty of Medical Sciences, Matsuoka-cho, YoshIDa-gun, Fukui 910-1193, Japan

Thomas F Baumert, Dr
Department of Medicine II, University of Freiburg, Hugstetter Strasse 55, D-79106 Freiburg, Germany

Stefano Bellentani, Professor
Fondo Studio Malattie Fegato-ONLUS, Sezione di Campogalliano, Via R. Luxemburg, 29/N, 41011 Campogalliano (MO), Italy

Luigi Bonavina, Professor
Department of Surgery, Policlinico San Donato, University of Milano, via Morandi 30, Milano 20097, Italy

Michael F Byrne, MD
Clinical Associate Professor
Division of Gastroenterology Vancouver General Hospital, 100-2647 Willow Street Vancouver BC V5Z 3P1, Canada

Patrice Philippe Cacoub, Professor
Department of Internal Medicine Hopital La Pitié Salpêtrière 41 Boulevard de l'hopital 75013 Paris, France

Wang-Xue Chen, Dr
Institute for Biological Sciences, National Research Concil Canada, 100 Sussex Drive, Room 3100, Ottawa, Ontario K1A 0R6, Canada

Francesco Costa, Dr
Dipartimento di Medicina Interna – U.O. di Gastroenterologia Università di Pisa – Via Roma, 67 – 56122 - Pisa, Italy

Bart Rik De Geest, Dr
Center for Molecular and Vascular Biology, Katholieke Universiteit Leuven, Campus Gasthuisberg, Herestraat 49, Leuven 3000, Belgium

Xue-Gong Fan, Professor
Department of Infectious Diseases, Xiangya Hospital, Central South University, Changsha 410008, China

Mitsuhiro Fujishiro, Dr
Department of Gastroenterology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan

Ghisetti Valeria Ghisetti, Dr
Laboratory of Microbiology, Molinette Hospital, Corso Bramante 88 / 90, 10126 Torino, Italy

Edoardo G Giannini, Assistant Professor
Department of Internal Medicine, Gastroenterology Unit, Viale Benedetto XV, no. 6, Genoa, 16132, Italy

Mario Guslandi, Professor
Department of Gastroenterology, S: Raffaele University Hospital, S: Raffaele University Hospitalvia Olgettina 60, Milano 20132, Italy

Johannes Herkel, Dr
I. Department of Medicine University Medical Centre Hamburg-Eppendorf Martinistr. 52,Hamburg 20246, Germany

Xiao-Long Ji, Professor
Institute of Nanomedicine, Chinese Armed Police General Hospital, 69 Yongding Road, Beijing 100039, China

Aydin Karabacakoglu, Dr, Assistant Professor
Department of Radiology, Meram Medical Faculty, Selcuk University, Konya 42080, Turkey

Serdar Karakose, Dr, Professor
Department of Radiology, Meram Medical Faculty, Selcuk University, Konya 42080, Turkey

Sherif M Karam, Dr
Department of Anatomy, Faculty of Medicine and Health Sciences, United Arab Emirates University, POBox17666, Al-Ain, United Arab Emirates

Zahariy Krastev, Professor
Department of Gastroneterology, Universiti Hospital "St. Ivan Rilski",#15, blvd "Acad. Ivan Geshov", Sofia 1431, Bulgaria

Shoji Kubo, MD
Hepato-Billiary-Pancreatic Surgery, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan

Ai-Ping Lu, Professor
China Academy of Traditional Chinese Medicine, Dongzhimen Nei, 18 Beixincang, Beijing 100700, China

Masatoshi Makuuchi, Professor
Department of Surgery, Graduate School of Medicine University of Tokyo, T Hepato-Biliary-Pancreatic Surgery Division Tokyo 113-8655, Japan

Reza Malekzadeh, Professor, Director
Digestive Disease Research Center, Tehran University of Medical Sciences, Shariati Hospital, Kargar Shomali Avenue, 19119 Tehran, Iran

Hiroshi Nakagawa, Assistant Professor
Gastroenterology Division, University of Pennsylvania, 415 Curie Blvd. 638BCRB, Philadelphia 19104, United States

Phillip S Oates, Dr
Department of Physiology, School of Biomedical and Chemical Sciences, The University of Western Australia, Perth, WA, Australia

Satoshi Osawa, MD
First Department of Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu, 431-3192, Japan

Bo-Rong Pan, Professor
Departmant of Oncology, Xijing Hospital, Fourth Military Medical University, No.1, F. 8, Bldg 10, 97 Changying East Road, Xi'an 710032, Shaanxi Province, China

Massimo Raimondo, Dr
Division of Gastroenterology and Hepatology, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, United States

Bernardino Rampone, Dr
Department of General Surgery and Surgical Oncology, University of Siena, viale Bracci, Siena 53100, Italy

Steffen Rickes, Dr
Department of Gastroenterology and Hepatology, University Hospital Magdeburg, Germany

Manuel Romero-Gómez, MD, Professor
Hepatology Unit, Hospital Universitario de Valme, Ctra de Cádiz s/n, Sevilla 41014, Spain

Francis Seow-Choen, Professor
Seow-Choen Colorectal Centre, Mt Elizabeth Medical Centre, Singapore, 3 Mt Elizabeth Medical Centre #09-10, 228510, Singapore

Ala Sharara, MD, FACP, Associate Professor, Head
Division of Gastroenterology, Department of Internal Medicine, American University of Beirut, Beirut, Lebanon

Michael Steer, Professor
Department of Surgery, Tufts-Nemc, 860 Washington St, Boston, Ma 02111, United States

Simon D Taylor-Robinson, MD
Department of Medicine A, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0HS, United Kingdom

Khalid Ahnini Tazi, PhD, Assistant Professor
Laboratoire d'Hemodynamique Splanchnique et de Biologie Vascilaire, Inserm, Hopital Beaujon., Clichy 92118, France

Nick Paul Thompson, MD
Department of Medicine, Freeman Hospital, Newcastle Upon Tyne, NE7 7DN, Unite Kingdom

Dino Vaira, Professor
Department of Internal Medicine and Gastroent, University of Bologna, S.Orsola-Malpighi Hospital - Nuove Patologie, Pad. 5 - via Massarenti 9, Bologna 40138, Italy

Siegfried Wagner, Professor
Medizinische Klinik II, Klinikum Deggendorf, Perlasberger Str. 41, Deggendorf 94469, Germany

Jesus K Yamamoto-Furusho, Dr
Gastroenterology, Instituto Nacional de Ciencias Medicas y Nutricion, Vasco de Quiroga 15, Col. seccion XVI, Mexico 14000, Mexico

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5589
World Journal of Gastroenterology ISSN 1007-9327

Meetings

MAJOR MEETINGS COMING UP

First Biennial Congress of the Asian-Pacific Hepato-Pancreato-Biliary Association
March 2007
Fukuoka, Japan
<http://www.congre.co.jp/1st-aphpba>/

American College of Gastroenterology Annual Scientific
20-25 October 2006
Las Vegas, NV

14th United European Gastroenterology Week, UEGW
21-25 October 2006
Berlin, Germany

APDW 2006: Asian Pacific Digestive Week 2006
26-29 November 2006
Lahug Cebu City, Philippines

EVENTS AND MEETINGS IN THE UPCOMING 6 MONTHS

Falk Symposium 151: Emerging Issues in Inflammatory Bowel Diseases
24-25 March 2006
Sydney - NSW
Falk Foundation e.V.
symposia@falkfoundation.de

10th International Congress of Obesity
3-8 September 2006
Sydney
Event Planners Australia
enquiries@ico2006.com
www.ico2006.com

Easl 2006 - the 41st annual
26-30 April 2006
Vienna, Austria
Kenes International

Prague hepatology 2006
14-16 September 2006
Prague
Foundation of the Czech Society of Hepatology
veronika.revicka@congressprague.cz
www.czech-hepatology.cz/phm2006

12th International Symposium on Viral Hepatitis and Liver Disease
1-5 July 2006
Paris
MCI France
isvhld2006@mci-group.com
www.isvhld2006.com

Falk Symposium 152: Intestinal Disease Part I, Endoscopy 2006 - Update and Live Demonstration
4-5 May 2006
Berlin
Falk Foundation e.V.
symposia@falkfoundation.de

Falk Symposium 153: Intestinal Disease Part II, Immunoregulation in Inflammatory Bowel Disease - Current Understanding and Innovation
6-7 May 2006
Berlin
Falk Foundation e.V.
symposia@falkfoundation.de

ILTS 12th Annual International Congress
3-6 May 2006
Milan
ILTS
www.ilts.org

Internal Medicine: Gastroenterology
22 July 2006-1 August 2006
Amsterdam
Continuing Education Inc
jbarnhart@continuingeducation.net

6th Annual Gastroenterology And Hepatology
15-18 March 2006
Rio Grande
Office of Continuing Medical Education
cmenet@jhmi.edu
www.hopkinscme.net

World Congress on Gastrointestinal Cancer
28 June 2006-1 July 2006
Barcelona, Spain
c.chase@imedex.com

International Conference on Surgical Infections, ICSI2006
6-8 September 2006
Stockholm
European Society of Clinical Microbiology and Infectious Diseases
icsi2006@stocon.se
www.icsi2006.se/9/23312.asp

7th World Congress of the International Hepato-Pancreato-Biliary Association
3-7 September 2006
Edinburgh
Edinburgh Convention Bureau
convention@edinburgh.org
www.edinburgh.org/conference

Society of American Gastrointestinal Endoscopic Surgeons
26-29 April 2006
Dallas - TX
www.sages.org

Digestive Disease Week 2006
20-25 May 2006
Los Angeles
www.ddw.org

Annual Postgraduate Course
25-26 May 2006
Los Angeles, CA
American Society of Gastrointestinal Endoscopy
www.asge.org/education

American Society of Colon and Rectal Surgeons
3-7 June 2006
Seatlle - Washington
www.fascrs.org

EVENTS AND MEETINGS IN 2006

10th World Congress of the International Society for Diseases of the Esophagus
22-25 February 2006
Adelaide
isde@sapmea.asn.au
www.isde.net

Falk Symposium 151: Emerging Issues in Inflammatory Bowel Diseases
24-25 March 2006
Sydney - NSW
Falk Foundation e.V.
symposia@falkfoundation.de

10th International Congress of Obesity
3-8 September 2006
Sydney
Event Planners Australia
enquiries@ico2006.com
www.ico2006.com

Easl 2006 - the 41st annual
26-30 April 2006
Vienna, Austria
Kenes International

VII Brazilian Digestive Disease Week
19-23 November 2006
www.gastro2006.com.br

International Gastrointestinal Fellows Initiative
22-24 February 2006
Banff, Alberta
Canadian Association of Gastroenterology
cagoffice@cag-acg.org
www.cag-acg.org

Canadian Digestive Disease Week
24-27 February 2006
Banff, Alberta
Digestive Disease Week Administration
cagoffice@cag-acg.org
www.cag-acg.org

Prague Hepatology 2006
14-16 September 2006
Prague
Foundation of the Czech Society of Hepatology
veronika.revicka@congressprague.cz
www.czech-hepatology.cz/phm2006

12th International Symposium on Viral Hepatitis and Liver Disease
1-5 July 2006
Paris
MCI France
isvhld2006@mci-group.com
www.isvhld2006.com/

Falk Seminar: XI Gastroenterology Seminar Week
4-8 February 2006
Titisee
Falk Foundation e.V.
symposia@falkfoundation.de

European Multidisciplinary Colorectal Cancer Congress 2006
12-14 February 2006
Berlin
Congresscare
info@congresscare.com
www.colorectal2006.org

Falk Symposium 152: Intestinal Disease Part I, Endoscopy 2006 - Update and Live Demonstration
4-5 May 2006
Berlin
Falk Foundation e.V.
symposia@falkfoundation.de

Falk Symposium 153: Intestinal Disease Part II, Immunoregulation in Inflammatory Bowel Disease - Current Understanding and Innovation
6-7 May 2006
Berlin
Falk Foundation e.V.
symposia@falkfoundation.de

14th United European Gastroenterology Week
21-25 October 2006
Berlin
United European Gastroenterology Federation
www.uegw2006.de

World Congress on Controversies in Obesity, Diabetes and Hypertension
25-28 October 2006
Berlin
comtec international
codhy@codhy.com
www.codhy.com

Asia Pacific Obesity Conclave
1-5 March 2006
New Delhi
info@apoc06.com
www.apoc06.com/

ILTS 12th Annual International Congress
3-6 May 2006
Milan
ILTS
www.ilts.org

XXX Panamerican Congress of Gastroenterology
11-16 November 2006
Cancun
www.panamericano2006.org.mx

Internal Medicine: Gastroenterology
22 July 2006-1 August 2006
Amsterdam
Continuing Education Inc
jbarnhart@continuingeducation.net

6th Annual Gastroenterology And Hepatology
15-18 March 2006
Rio Grande
Office of Continuing Medical Education
cmenet@jhmi.edu
www.hopkinscme.net

Hepatitis 2006
25 February 2006-5 March 2006
Dakar
hepatitis2006@mangosee.com
mangosee.com/mangosteen/hepatitis2006/hepatitis2006.htm

World Congress on Gastrointestinal Cancer
28 June 2006-1 July 2006
Barcelona, Spain
c.chase@imedex.com

International Conference on Surgical Infections, ICSI2006
6-8 September 2006
Stockholm
European Society of Clinical Microbiology and Infectious Diseases
icsi2006@stocon.se
www.icsi2006.se/9/23312.asp

5th International Congress of The African Middle East Association of Gastroenterology
24-26 February 2006
Sharjah
InfoMed Events
infoevent@infomedweb.com
www.infomedweb.com

7th World Congress of the International Hepato-Pancreato-Biliary Association
3-7 September 2006
Edinburgh
Edinburgh Convention Bureau
convention@edinburgh.org
www.edinburgh.org/conference

13th International Symposium on Pancreatic & Biliary Endoscopy
20-23 January 2006
Los Angeles - CA
laner@cshs.org

2006 Gastrointestinal Cancers Symposium
26-28 January 2006
San Francisco - CA
Gastrointestinal Cancers Symposium
Registration Center
giregistration@jspargo.com

Society of American Gastrointestinal Endoscopic Surgeons
26-29 April 2006
Dallas - TX
www.sages.org

Digestive Disease Week 2006
20-25 May 2006
Los Angeles
www.ddw.org

Annual Postgraduate Course
25-26 May 2006
Los Angeles, CA
American Society of Gastrointestinal Endoscopy
www.asge.org/education

American Society of Colon and Rectal Surgeons
3-7 June 2006
Seatlle - Washington
www.fascrs.org

71st ACG Annual Scientific and Postgraduate Course
20-25 October 2006
Venetian Hotel, Las Vegas, Neveda
The American College of Gastroenterology

AASLD 57th Annual - The Liver Meeting™
27-31 October 2006
Boston, MA
AASLD

New York Society for Gastrointestinal Endoscopy
13-16 December 2006
New York
www.nysge.org

EVENTS AND MEETINGS IN 2007

9th World Congress on Gastrointestinal Cancer
20-23 June 2007
Barcelona
Imedex
meetings@imedex.com

Gastro 2009, World Congress of Gastroenterology and Endoscopy London, United Kingdom 2009

PO Box 2345, Beijing 100023, China
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2006 September 14; 12(34): 5590-5592
World Journal of Gastroenterology ISSN 1007-9327

Instructions to authors

GENERAL INFORMATION

World Journal of Gastroenterology (*WJG, World J Gastroenterol* ISSN 1007-9327 CN 14-1219/R) is a weekly journal of more than 48 000 circulation, published on the 7th, 14th, 21st and 28th of every month.

Original Research, Clinical Trials, Reviews, Comments, and Case Reports in esophageal cancer, gastric cancer, colon cancer, liver cancer, viral liver diseases, *etc.*, from all over the world are welcome on the condition that they have not been published previously and have not been submitted simultaneously elsewhere.

Indexed and abstracted in

Current Contents/Clinical Medicine and Science Citation Index-Expanded, *Index Medicus*, MEDLINE and PubMed, Chemical Abstracts, EMBASE/Excerpta Medica, Abstracts Journals, Nature Clinical Practice Gastroenterology and Hepatology, CAB Abstracts and Global Health.ISI JCR 2003-2000 IF: 3.318, 2.532, 1.445 and 0.993.

Published by

The WJG Press

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed double-spaced on A4 (297 mm × 210 mm) white paper with outer margins of 2.5 cm. Number all pages consecutively, and start each of the following sections on a new page: Title Page , Abstract, Introduction, Materials and Methods, Results, Discussion, acknowledgements, References, Tables, Figures and Figure Legends. Neither the editors nor the Publisher is responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of The WJG Press, and may not be reproduced by any means, in whole or in part without the written permission of both the authors and the Publisher. We reserve the right to put onto our website and copy-edit accepted manuscripts. Authors should also follow the guidelines for the care and use of laboratory animals of their institution or national animal welfare committee.

Authors should retain one copy of the text, tables, photographs and illustrations, as rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for the loss or damage to photographs and illustrations in mailing process.

Online submission

Online submission is strongly advised. Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/index.jsp. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/wjg/help/instructions.jsp) before attempting to submit online. Authors encountering problems with the Online Submission System may send an email you describing the problem to wjg@wjgnet.com for assistance. If you submit your manuscript online, do not make a postal contribution. A repeated online submission for the same manuscript is strictly prohibited.

Postal submission

Send 3 duplicate hard copies of the full-text manuscript typed double-spaced on A4 (297 mm × 210 mm) white paper together with any original photographs or illustrations and a 3.5 inch computer diskette or CD-ROM containing an electronic copy of the manuscript including all the figures, graphs and tables in native Microsoft Word format or *.rtf format to:

Editorial Office

World Journal of Gastroenterology
Editorial Department: Apartment 1066, Yishou Garden,
58 North Langxinzhuang Road,
PO Box 2345, Beijing 100023, China
E-mail: wjg@wjgnet.com
http: //www.wjgnet.com
Telephone: +86-10-85381901
Fax: +86-10-85381893

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using a word-processing software. All submissions must be typed in 1.5 line spacing and in word size 12 with ample margins. The letter font is Tahoma. For authors from China, one copy of the Chinese translation of the manuscript is also required (excluding references). Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Full manuscript title, running title, all author(s) name(s), affiliations, institution(s) and/or department(s) where the work was accomplished, disclosure of any financial support for the research, and the name, full address, telephone and fax numbers and email address of the corresponding author should be included. Titles should be concise and informative (removing all unnecessary words), emphasize what is new, and avoid abbreviations. A short running title of less than 40 letters should be provided. List the author(s)' name(s) as follows: initial and/or first name, middle name or initial(s) and full family name.

Abstract

An informative, structured abstract of no more than 250 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections: AIM: Only the purpose should be included. METHODS: The materials, techniques, instruments and equipments, and the experimental procedures should be included. RESULTS: The observatory and experimental results, including data, effects, outcome, *etc.* should be included. Authors should present *P* value where necessary, and the significant data should accompany. CONCLUSION: Accurate view and the value of the results should be included.

The format of structured abstracts is at: http://www.wjgnet.com/wjg/help/11.doc

Key words

Please list 6-10 key words that could reflect content of the study mainly from *Index Medicus*.

Text

For most article types, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include in appropriate Figures and Tables. Data should be presented in the body text or in Figures and Tables, but not in both.

Illustrations

Figures should be numbered as 1, 2, 3 and so on, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. No detailed legend should be involved under the figures. This part should be added into the text where the figures are applicable. Digital images: black and white photographs should be scanned and saved in TIFF format at a resolution of 300 dpi; color images should be saved as CMYK (print files) but not as RGB (screen-viewing files). Place each photograph in a separate file. Print images: supply images of size no smaller than 126 mm × 76 mm printed on smooth surface paper; label the image by writing the Figure number and orientation using an arrow. Photomicrographs: indicate the original magnification and stain in the legend. Digital Drawings: supply files in EPS if created by freehand and illustrator, or TIFF from photoshops. EPS files must be accompanied by a version in native file format for editing purposes. Existing line drawings should be scanned at a resolution of 1200 dpi and as close as possible to the size where they will appear when printed. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...

Tables

Three-line tables should be numbered as 1, 2, 3 and so on, and mentioned clearly in the main text. Provide a brief title for each table. No detailed legend should be included under the tables. This part should be added into the text where the tables are applicable. The information should complement but not duplicate that contained in the text. Use one horizontal line under the title, a second under the column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P<0.05$, $^{b}P<0.01$ should be noted ($P>0.05$ should not be noted). If there are other series of *P* values, $^{c}P<0.05$ and $^{d}P<0.01$ are used. Third series of *P* values can be expressed as $^{e}P<0.05$ and $^{f}P<0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or some other symbols with

a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.* in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscripts and who endorse the data and conclusions are included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should code the references according the citation order in text in Arabic numerals, put references codes in square brackets, superscript it at the end of citation content or the author name of the citation. For those citation content as the narrate part, the coding number and square brackets should be typeset normally. For example, Crohn's disease (CD) is associated with increased intestinal permeability[1,2]. If references are directly cited in the text, they would be put together with the text, for example, from references [19,22-24], we know that...

When the authors code the references, please ensure that the order in text is the same as in reference part and also insure the spelling accuracy of the first author's name. Do not code the same citation twice.

PMID requirement

PMID roots in the abstract serial number indexed by PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed). The author should supply the PMID for journal citation. For those references that have not been indexed by PubMed, a printed copy of the first page of the full reference should be submitted.

The accuracy of the information of the journal citations is very important. Through reference testing system (http://www.wjgnet.com/cgi-bin/index.pl), the authors and editor could check the authors name, title, journal title, publication date, volume number, start page, and end page. We will interlink all references with PubMed in ASP file so that the readers can read the abstract of the citations online immediately.

Style for journal references

Authors: the first author should be typed in bold-faced letter. The surname of all authors should be typed with the initial letter capitalized and followed by their name in abbreviation (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). Title of the cited article and italicized journal title (Journal title should be in its abbreviation form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634]

Note: The author should test the references through reference testing system (http://www.aushome.cn/cgi-bin/index.pl)

Style for book references

Authors: the first author should be typed in bold-faced letter. The surname of all authors should be typed with the initial letter capitalized and followed by their name in abbreviation (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Grover VP**, Dresner MA, Forton DM, Counsell S, Larkman DJ, Patel N, Thomas HC, Taylor-Robinson SD. Current and future applications of magnetic resonance imaging and spectroscopy of the brain in hepatic encephalopathy. *World J Gastroenterol* 2006; **12**: 2969-2978 [PMID: 16718775]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci U S A* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900]

No volume or issue

9 Outreach: bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ Cell Tumour Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http//www.cdc.gov/ncidod/EID/eid.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Inappropriate references

Authors should always cite references that are relevant to their article, and avoid any inappropriate references. Inappropriate references include those that are linked with a hyphen and the difference between the two numbers at two sides of the hyphen is more than 5. For example, [1-6], [2-14] and [1, 3, 4-10, 22] are all considered as inappropriate references. Authors should not cite their own unrelated published articles.

Statistical data

Present as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as γ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, *p*(B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, ρ (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2 not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format about how to accurately write common units and quantum is at: http://www.wjgnet.com/wjg/help/15.doc

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further mention.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyr*A, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H pylori, E coli, etc.*

SUBMISSION OF THE REVISED MANUSCRIPTS AFTER ACCEPTED

Please revise your article according to the revision policies of *WJG*. The revised version including manuscript and high-resolution image figures (if any) should be copied on a floppy or compact disk. Author should send the revised manuscript, along with printed high-resolution color or black and white photos, copyright transfer letter, the final check list for authors, and responses to reviewers by a courier (such as EMS) (submission of revised manuscript by e-mail or on the *WJG* Editorial Office Online System is NOT available at present).

Language evaluation

The language of a manuscript will be graded before sending for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing; (4) Grade D: rejected. The revised articles should be in grade B or grade A.

Copyright assignment form

Please downloaded CAF from http://www.wjgnet.com/wjg/help/9.doc.

We certify that the material contained in this manuscript:

Ms:

Title:

is original, except when appropriately referenced to other sources, and that written permission has been granted by any existing copyright holders. We agree to transfer to WJG all rights of our manuscript, including: (1) all copyright ownership in all print and electronic formats; (2) the right to grant permission to republish or reprint the stated material in whole or in part, with or without a fee; (3) the right to print copies for free distribution or sale; (4) the right to republish the stated material in a collection of articles or in any other format. We also agree that our article be put on the Internet.

Criteria for authorship: The WJG requests and publishes information about contributions of each author named to the submitted study. Authorship credit should be based on (1)direct participation in the study, including substantial contributions to conception and design of study, or acquisition of data, or analysis and interpretation of data; (2) manuscript writing, including drafting the article, or revising it critically for important intellectual content; (3)supportive work, including statistical analysis of data, or acquisition of funding, or administration, technology and materials support, or supervision, or supportive contributions. Authors should meet at least one of the three conditions. The WJG does not publish co-first authors and co-corresponding authors.

We hereby assign copyright transfer to WJG if this paper is accepted.

Author Name in full (Full names should be provided, with first name first, followed by middle names and family name at the last, eg, Eamonn MM Quigley). Handwritten names are not accepted.

Author Name in abbreviation (Family name is put first in full, followed by middle names and first name in abbreviation with first letter in capital, eg, Quigley EMM). Handwritten names are not accepted.

1 Full Name: _____

Abbreviation Name: _____

Signed: _____

Date: _____

2 Full Name: _____

Abbreviation Name: _____

Signed: _____

Date: _____

3 Full Name: _____

Abbreviation Name: _____

Signed: _____

Date: _____

4 Full Name: _____

Abbreviation Name: _____

Signed: _____

Date: _____

5 Full Name: _____

Abbreviation Name: _____

Signed: _____

Date: _____

6 Full Name: _____

Abbreviation Name: _____

Signed: _____

Date: _____

7 Full Name: _____

Abbreviation Name: _____

Signed: _____

Date: _____

8 Full Name: _____

Abbreviation Name: _____

Signed: _____

Date: _____

9 Full Name: _____

Abbreviation Name: _____

Signed: _____

Date: _____

10 Full Name: _____

Abbreviation Name: _____

Signed: _____

Date: _____

Final check list for authors

The format is at: http://www.wjgnet.com/wjg/help/13.doc

Responses to reviewers

Please revise your article according to the comments/suggestions of reviewers. The format for responses to the reviewers' comments is at: http://www.wjgnet.com/wjg/help/10.doc

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Publication fee

Authors of accepted articles must pay publication fee.
EDITORIAL and LETTERS TO THE EDITOR are free of change.