World Journal of Gastroenterology®

Volume 13 Number 38
October 14, 2007

National Journal Award
2005

Baishideng

Editorial Department of *World Journal of Gastroenterology*
77 Shuangta Xijie, Taiyuan 030001, Shanxi Province, China
Telephone: +86-351-4078656
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 13 Number 38 Oct 14 2007

ISSN 1007-9327
CN 14-1219/R

World Journal of Gastroenterology®

Indexed and Abstracted in:

Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®) and Journal Citation Reports/Science Edition, *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 13 Number 38
October 14, 2007
World J Gastroenterol
2007 October 14; 13(38): 5043-5168

Online Submissions
wjg.wjgnet.com
www.wjgnet.com
Printed on Acid-free Paper
世界胃肠病学杂志

A Weekly Journal of Gastroenterology and Hepatology

National Journal Award
2005

World Journal of Gastroenterology®

Weekly Established in October 1995

Volume 13 Number 38
October 14, 2007

Baishideng

Contents

Contents

World Journal of Gastroenterology
Volume 13 **Number** 38 **October** 14, 2007

World Journal of Gastroenterology
Volume 13 **Number** 38 **October** 14, 2007

Contents

Responsible E-Editor for this issue: Hai-Feng Wang

C-Editor for this issue: Dr. Daniel Worthley

Responsible S-Editor for this issue: Ye Liu

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, Science Citation Index Expanded (also known as SciSearch®) and Journal Citation Reports/Science Edition, *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 *WJG*. 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.

NAME OF JOURNAL
World Journal of Gastroenterology

RESPONSIBLE INSTITUTION
Department of Science and Technology of Shanxi Province

SPONSOR
Taiyuan Research and Treatment Center for Digestive Diseases, Taiyuan 77, Shuangta Xijie, Taiyuan 030001, Shanxi Province, China

EDITING
Editorial Board of *World Journal of Gastroenterology,* 77 Shuangta Xijie, Taiyuan 030001,
Shanxi Province, China
Telephone: +86-351-4078656
E-mail: wjg@wjgnet.com

PUBLISHING
Editorial Department of *World Journal of Gastroenterology,* 77 Shuangta Xijie, Taiyuan 030001,
Shanxi Province, China
Telephone: +86-351-4078656
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PRINTING
Beijing Kexin Printing House

OVERSEAS DISTRIBUTOR
Beijing Bureau for Distribution of Newspapers and Journals
(Code No. 82-261)
China International Book Trading Corporation PO Box 399, Beijing, China (Code No. M4481)

PUBLICATION DATE
October 14, 2007

EDITOR-IN -CHIEF
Lian-Sheng Ma, *Taiyuan*

SUBSCRIPTION
RMB 50 Yuan for each issue, RMB 2400 Yuan for one year

CSSN
ISSN 1007-9327
CN 14-1219/R

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.

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5043-5051
World Journal of Gastroenterology ISSN 1007-9327

EDITORIAL

Severe acute pancreatitis: Clinical course and management

Hans G Beger, Bettina M Rau

Hans G Beger, Department of General Surgery, Department of Viszeralsurgery, University of Ulm (1982-2001), Donau-Klinikum, Neu-Ulm, Germany
Bettina M Rau, Department of General, Viszeral, Vascular and Pediatric Surgery, Universitätsklinikum des Saarlandes, Homburg/Saar, Germany
Correspondence to: Hans G Beger, MD, c/o University Hospital, Steinhoevelstr. 9, D-89075 Ulm, Germany. hans.beger@medizin.uni-ulm.de
Telephone: +49-731-50069420 Fax: +49-731-50069421
Received: March 13, 2007 Revised: August 11, 2007

Key words: Severe acute pancreatitis; Multiorgan failure syndrome; Infected necrosis; Fluid replacement; Enteral feeding; Surgical and interventional debridement

Beger HG, Rau BM. Severe acute pancreatitis: Clinical course and management. *World J Gastroenterol* 2007; 13(38): 5043-5051

http://www.wjgnet.com/1007-9327/13/5043.asp

Abstract

Severe acute pancreatitis (SAP) develops in about 25% of patients with acute pancreatitis (AP). Severity of AP is linked to the presence of systemic organ dysfunctions and/or necrotizing pancreatitis pathomorphologically. Risk factors determining independently the outcome of SAP are early multi-organ failure, infection of necrosis and extended necrosis (> 50%). Up to one third of patients with necrotizing pancreatitis develop in the late course infection of necroses. Morbidity of SAP is biphasic, in the first week strongly related to early and persistence of organ or multi-organ dysfunction. Clinical sepsis caused by infected necrosis leading to multi-organ failure syndrome (MOFS) occurs in the later course after the first week. To predict sepsis, MOFS or deaths in the first 48-72 h, the highest predictive accuracy has been objectified for procalcitonin and IL-8; the Sepsis-Related Organ Failure Assessment (SOFA)-score predicts the outcome in the first 48 h, and provides a daily assessment of treatment response with a high positive predictive value. Contrast-enhanced CT provides the highest diagnostic accuracy for necrotizing pancreatitis when performed after the first week of disease. Patients who suffer early organ dysfunctions or at risk of developing a severe disease require early intensive care treatment. Early vigorous intravenous fluid replacement is of foremost importance. The goal is to decrease the hematocrit or restore normal cardiocirculatory functions. Antibiotic prophylaxis has not been shown as an effective preventive treatment. Early enteral feeding is based on a high level of evidence, resulting in a reduction of local and systemic infection. Patients suffering infected necrosis causing clinical sepsis, pancreatic abscess or surgical acute abdomen are candidates for early intervention. Hospital mortality of SAP after interventional or surgical debridement has decreased in high volume centers to below 20%.

INTRODUCTION

Dealing with the clinical course of acute pancreatitis (AP) and the management of severe acute pancreatitis (SAP) are complicated by limited understanding of pathogenesis and multi-causality of the disease, uncertainties to predict outcome and a few effective treatment modalities. AP comprises clinically a mild oedematous-interstitial inflammation, which is a self-limiting disease, and a severe type of AP with a local necrotizing inflammation and systemic complications. Despite the importance of recognizing severe disease early in the course, many patients initially identified as having mild disease, progress to severe pancreatitis over the initial period of disease. Clinical studies and experimental new data have led to considerable progress in understanding the pathophysiological events of the early period of human AP, but the underlying processes leading to acinus cell necroses and the propagation of the necrotizing inflammation by impaired microcirculation of pancreatic tissue compartments in the initial 48-72 h, are still unknown to a large extent. Hence, management of human AP has been empiric and conflicting opinions are still present regarding medical and surgical management concepts.

PATTERN OF INFLAMMATION

The tissue response of the pancreas to an injury like acinus cell necrosis leads to production and liberation of proinflammatory cytokines, chemokines and other biological active compounds[1-4]. Clinical and experimental studies have verified activation of local macrophages and attraction of activated polymorphonuclear cells (PMNs) as first-line players in the defense and limitation of pancreatic tissue injury[5-7]. Inflammatory mediators are primarily released from the splanchnic area and gain access to the systemic compartment mainly by lymphatic, portal vein and suprahepatic circulation[1,8]. The lungs are the first

Table 1 Severe acute pancreatitis: Gut barrier dysfunction causes local changes and systemic complications

Local	Systemic consequences
Mucosal ischemia[10,15,16]	Priming of neutrophils[20-22]
Disruption of mucosal epithelial integrity[17]	Endotoxemia[14,23,24]
Reperfusion injury of mucosal epithelia[18]	Bacterial translocation[25-27]
Increase of intestinal permeability[19]	Cytokine overproduction[1,2,28]
Gram-negative intestinal bacterial overgrowth[11]	Impaired systemic immunity[29,30]
Impaired mucosal immunity[11]	

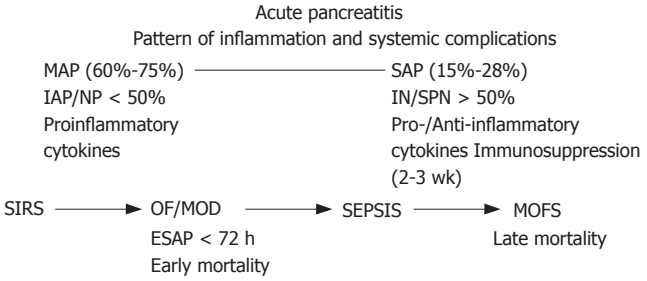


Figure 1 Pattern of inflammation and systemic complications in acute pancreatitis. MAP: Mild acute pancreatitis; SAP: Severe acute pancreatitis; IAP: Intestinal oedematous pancreatitis; NP: Necrotizing pancreatitis; IN: Infected necrosis; SPN: sterile pancreatic necrosis; SIRS: severe inflammatory response syndrome; OF: Organ failure; MOD: Multiorgan failure; SEPSIS: Leukocytes > 10 000/mm^3 + fever > 38.5 rectal/> 48 h + metabolic acidosis base excess > -4 mmol/L; MOFS: Multiorgan failure syndrome; ESAP: Early severe acute pancreatitis.

pass taker of the porto-hepatic blood and lymph of the splanchnic compartments enriched of activated PMNs, cytokines and other biological active compounds. Gut barrier failure, with the ensuing translocation of bacteria and endotoxin, has been proposed as a major contributor to the development of local infection and multi-organ failure in SAP[9]. Evidence of the association between gut injury and the subsequent development of infected necroses and distant organ failure continues to increase. Intestinal permeability disturbances have been found in humans with SAP 72 h after onset, correlating strongly with clinical outcome[10]: the increase of permeability was significantly higher in patients who developed multi-organ failure and/or died compared to patients suffering from mild attacks[11]. Intestinal permeability increases gradually during the course of SAP reaching a maximum at the end of the first week[12]. In a recent prospective study of patients with AP, endotoxemia as a consequence of increase of gut permeability was found on the day of admission to the hospital significantly more common and of greater magnitude in severe attacks than in those with mild attacks, in non-survivors than in survivors, and in patients who developed multi-organ failure than in those who did not[13,14] (Table 1).

The peritoneal compartment is the site of pro-inflammatory reaction to pancreatic necrosis, whereas an anti-inflammatory response dominates in the lymph collected from the thoracic duct, as well as in the systemic circulation during the first week after onset of systemic complications[1]. The cytokine levels in the blood and lymph are closely associated with the severity of illness on admission, the magnitude of multi-organ failure syndrome (MOFS) and the outcome as well[1,2]. Correlated with local and systemic complications, a compartmentalization of the inflammatory responses has been objectified. Local inflammatory cytokines at high concentrations are found in the portal and splanchnic circulation at the same time when in the systemic blood compartment anti-inflammatory compounds prevail over inflammatory cytokines. In SAP, a compensatory anti-inflammatory response affects the immunocompetence[30,31]. Patients with SAP show impaired immune response in regard to reduced HLA-DR expression of monocytes and macrophages[29,32], reduced numbers of CD4- and CD8-positive T-cells[33,34], an impairment of mononuclear phagocytic capacity[35] and an increase of the anti-inflammatory cytokine IL-10 and IL-1 receptor antagonist[36]. Reduced immune competence, as objectified by reduced expression of HLA-DR of monocytes and macrophages, predicts the development of organ failure and is associated with secondary infection[29] (Figure 1).

CLASSIFICATION OF ACUTE PANCREATITIS

At the beginning of the 1980s, the morphologic feature of SAP was established by the definition of infected and sterile necrosis, pancreatic abscess and postacute pseudocysts as the principal morphologic and bacteriologic criteria for clinical severity and considered to be determinants of the clinical course[37]. Macroscopically, necrotizing pancreatitis is characterized by focal or diffuse areas of devitalized parenchyma frequently associated with peripancreatic fatty tissue necroses extending sometimes to retroperitoneal spaces up to the pelvis. Intrapancreatic hemorrhage is variably present and may lead to an acute abdominal compartment syndrome. Infection of necrosis occurs in up to 30% of all patients with necrotizing pancreatitis[27,38]. In the clinical setting of AP, post-acute pseudocysts and pancreatic abscess are late consequences of the disease[39]. In both subgroups of AP, an inflammatory wall is developed which separates the inflammatory processes from the surrounding tissues. Both features have differences in clinical symptomatology and associated morbidity. Peripancreatic fluid collection that arises early during the course of AP is frequently a sign of severity. However, in most instances peripancreatic fluid collection disappears without any treatment[40].

The Atlanta Classification is accepted worldwide as the first clinical reliable classification system of AP. But the accumulation of clinical data forces a revision of the Atlanta criteria of severity. Organ failure has been recognized as a more important determinant of survival than the extent of pancreatic necroses. Particularly early multi-organ failure at admission or in the first days predicts strongly the clinical course and the outcome. Severity of organ failure using multi-step criteria as introduced for septic patients by the SOFA-score is considered clinically relevant and increasingly applied for severity scoring and predicting outcome[41]. The SOFA criteria for systemic

Table 2 Clinical course of AP/SAP

	Clinical	Pathophysiologic process
Early: d 1-10 after HA ESAP in about 20% of SAP	Hypovolemia Abdominal pain Dysfunction Pulmonary Renal Cardiocirculatory Liver Intestine	Fluid sequestration Liberation of pro- and anti-inflammatory cytokines Endotoxemia Liberation of vasoactive substances Disturbance of blood coagulation Translocation of endotoxin and bacteria
Late > 2 wk after HA	Local and systemic septic complications IN, SPN	Bacterial translocation CARS Anti-inflammatory reaction Immunosuppression

AP: Acute pancreatitis; SAP: Severe acute pancreatitis; ESAP: Early severe acute pancreatitis; HA: Hospital admission; IN: Infected necrosis; SPN: Sterile pancreatic necrosis; CARS: Compensatory antiinflammatory syndrome.

organ dysfunctions are clinically more reliable for decision making than the Atlanta criteria.

CLINICAL COURSE OF SAP

Acute pancreatitis is not a stable disease. Increasing amounts of intrapancreatic and retroperitoneal necroses are closely related to the frequency and severity of local and systemic complications[27]. About 70%-80% of AP takes a mild course and is associated only with minimal organ dysfunctions. First clinical signs are abdominal pain located in the epigastrium, frequently radiating into the midback (Table 2). Clinical improvement can easily be achieved by fluid replacement, a pain treatment and re-institution of regular food intake. The initial 2-4 d after onset of symptoms are most important, when about 15%-25% of patients with AP take the course of a severe disease. Based on clinical and experimental data, this period is characterized by an initial hypovolaemic state[42-44]. In SAP, hypotension or even shock occurs as a consequence of sequestration of protein-rich fluids into the pancreas, the retroperitoneal spaces and the abdominal cavity. The initial systemic inflammatory response syndrome causes a hyperinflammatory reaction exerting systemic organ dysfunctions of the lungs, kidneys, cardiocirculatory system and splanchnic intestinal compartments[45,46]. Acute fluid collections arise early in the course of severe acute pancreatitis, lack a well-defined wall and are usually peripancreatic in location, and usually resolve without sequelae but may evolve into pancreatic pseudocysts or abscesses. Acute fluid collections rarely require drainage. About 60%-70% of fluid collection resolves spontaneously and has no connection with the pancreatic duct system[47].

EARLY SEVERE ACUTE PANCREATITIS

About 20% of patients with SAP develop in the 72 h after onset of the disease organ failure or even have organ- or multi-organ failure at admission to hospitals[48]. Despite application of maximum intensive care treatment, 30%-50% of the patients with early severe acute pancreatitis do not promptly respond to ICU treatment and take a complicated course with persistence of multi-system organ dysfunctions. Patients suffering from early and persistent multi-organ insufficiency syndrome have a high risk of mortality[49]. Recently it has been shown that severe organ failure within the first week after onset of AP before any kind of intervention is closely linked to clinically relevant pancreatic infection which occurs two weeks later[50]. Early multi-organ dysfunction syndrome (MODS) obviously triggers additional mechanisms that render bacterial translocation into clinically manifested sepsis. Early onset MODS > 2 organs has proved to be the predominant risk factor for death. Early mortality in the first 6-10 d of SAP is caused by severe inflammatory response syndrome (SIRS) associated with early multi-organ insufficiency syndrome (Table 3). Early mortality was reported between 42% and 60%[51-53].

Table 3 Severe acute pancreatitis-early organ failure

Admission			Dynamic of organ failure	Hospital mortality
ESAP (n = 47)	SOF	25 (53%)	Reversible 9 develop MOF 14 (30%)	42%
	MOF	22 (47%)	Reversible 1 progress to MOFS 21 (95%)	
SAP (n = 111)	OF (-)	30 (27%)		
	SOF	26 (23%)		14%
	MOF	55 (50%)		

ESAP: Early severe acute pancreatitis; SOF: Single organ failure; MOF: Multiorgan failure; SAP: Severe acute pancreatitis; OF: Organ failure. Reversibility or assistance in spite of maximum intensive care treatment of early organ failure or early organ failure syndrome (n = 158)[48].

Presence of necrosis and infection

Gross destruction of the pancreatic gland by tissue necroses is observed in about 20% of patients with AP and takes place in the first week after onset[54]. Experimental and clinical observations reveal that development of pancreatic necrosis is accompanied by an increase of local and systemic organ complications, increasing the risk of morbidity and mortality compared to patients with interstitial-oedematous pancreatitis[55,56] (Figure 2). Most patients who develop early or late organ failure suffer from necrotizing pancreatitis. Autopsy data and surgical results have verified that more than 80% of deaths are correlated with the presence of necroses. The highest risk for local and systemic complications is seen in patients who show extended necrosis of more than ⩾ 50% of the pancreas by magnetic resonance tomography (MRT) or contrast-enhanced computer tomography (CECT)[57,58]. Patients with sterile extended pancreatic necroses (> 50%) display clinically signs of sepsis including organ dysfunctions, septic fever, leucocytosis, hyperdynamic cardiocirculatory state and intestinal motility disorder. To discriminate clinical infection from sterile necrosis, the use of sepsis criteria is not reliable in patients with extended sterile necrosis.

In addition to the presence of pancreatic parenchymal necrosis, the occurrence and extent of the necrotizing process into extrapancreatic retroperitoneal fatty tissue

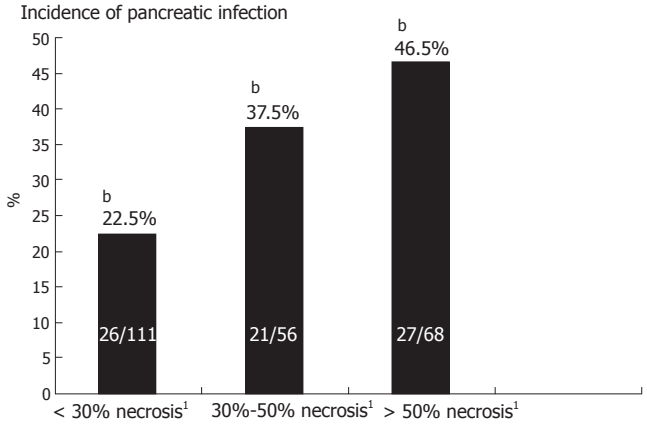


Figure 2 Relation between bacterial infection and extent of necrosis in 225 patients with severe acute pancreatitis. [1]On the basis of contrast-enhanced CT. [b]P = 0.008 between the groups, Cochran-Armitage trend test.

spaces including tissue compartments of the mesentery of the small and large bowel, the peri-renal fat and the para- and retrocolic compartments, are important factors influencing the course of the disease and strongly affect the clinical severity[59]. The overall infection rate of pancreatic tissue in necrotizing pancreatitis is up to 30% and may increase to 70% in the 3rd wk[27] (Table 4).

The setting of pancreatic infection includes infected necrosis, pancreatic abscess and infected pancreatic pseudocysts. The bacteriological analysis of puncture aspirates or of intraoperative smears reveals predominantly gram-negative microbes deriving from the intestine. *Escherichia coli* was the most frequent pathogen followed by *Enterococcus* and *Klebsiella*[27]. However, in recent years a shift of the bacterial pattern has been observed towards more gram-positive bacteria like *Staph aureus* and *Enterobacteriaceae*[60,61]. The presence of candida species in infected necroses has been observed in 5%-15%[62]. Candida patients have a higher mortality and experience more systemic complications than patients without candida infections of necroses. Recent data about the routine use of prophylactic antibiotics provided evidence that application of antibiotics contributes to the development of candida infection and to changes in bacterial spectrum of infected necroses with an increased incidence of gram-positive infections[62,63].

CT-guided fine needle aspiration (FNA) of the necrotic area is a safe procedure to diagnose infection, identify bacteria and institute appropriate therapy. To distinguish pancreatic inflammation from secondary infection, gram staining and culture must be performed after guided aspiration[64]. The knowledge of the bacteria and candida species and the pattern of chemo-resistance may lead to a rational antibiotic treatment.

MANAGEMENT OF SAP: PREDICTION OF SEVERITY AND OUTCOME

The management of patients with AP is challenging due to late hospitalization after onset of the acute attack and difficulty in distinguishing mild from severe disease in the first 48-72 h. Identification of risk factors for the development of necrotizing pancreatitis within the initial 24 h of hospitalization is of potential clinical importance. Patients who display at admission organ dysfunctions or an Apache Ⅱ score ⩾ 8[65] or C-reactive protein (CRP) > 120 mg/dL[66] or procalcitonin > 1.8 ng/mL[67] or a hematocrit > 44[44] should have early intensive care for optimal surveillance and ICU treatment. The use of early CECT or MRI for determination of severity is limited by several factors: (a) only a quarter of patients with acute pancreatitis develop necroses; (b) pancreatic necroses may not develop in the first 48 h; and (c) the presence of pancreatic necroses and the amount of necroses does not strongly correlate with the development of organ failure (Figure 3)[68]. The CECT based Balthazar classification shows the highest diagnostic and predictive accuracy when performed after the first week of disease. The APACHE Ⅱ scoring and the sequential Sepsis-Related Organ Failure Assessment (SOFA) have a highly reliable sensitivity and specificity and positive predictive value for the degree of severity of SAP. APACHE Ⅱ-, Marshall- and SOFA score can objectify the responses of the patients to intensive care measures (Table 5) on a daily basis. The biochemical parameters, CRP, procalcitonin and IL-8 have a high predictive accuracy for the degree of severity of necrotising pancreatitis in the first days. Procalcitonin > 1.4 ng/mL has a diagnostic accuracy of 70% for infection of necrosis; and procalcitonin of > 3.8 ng/mL predicts MODS with a diagnostic accuracy of 92%[71] (Table 6).

Table 4 Frequency of pancreatic infection in 427 patients[1] with necrotizing pancreatitis[2]

		NP (%)	AP (%)
Infected necrosis	99	23.2	6.9
Pancreatic abscess	40	9.4	2.8
Infected pseudocyst after AP	7	1.6	0.5
Total	146	34.2	10.1

[1]Pancreatic necrosis/extrapancreatic fatty tissue necrosis, pancreatic abscess, postacute pseudocyst. [2]5/1982-12/1996 Department of General Surgery, University of Ulm.

First line treatment of SAP

Admission hematocrit of > 47 and a failure of admission hematocrit to decrease at 24 h has been identified as reliable criteria of hemoconcentration of SAP in the very early period of the disease[42-44]. Vigorous intravenous fluid resuscitation is required to overcome systemic hypovolemia caused by intravascular fluid loss[72,73]. Intravenous fluid substitution for patients with predicted SAP should be established with 250-350 mL/h for the first 48 h[42]. Restoration of normal cardiocirculatory functions objectified by heart-rate, systolic or mean arterial blood pressure, an oxygen saturation of venous blood of > 95%, absence of a base deficit > 5 μmol/L and urine flow of ⩾ 50 mL/h are decisive criteria of treatment response.

In mild biliary acute pancreatitis, endoscopic retrograde cholangiopancreatography (ERCP) and removal of common bile duct stones do not change the natural course

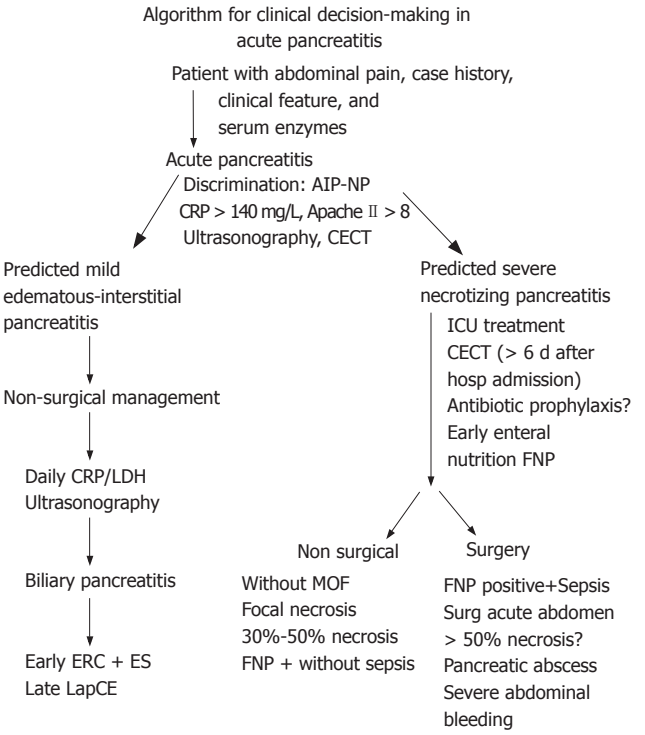


Figure 3 Algorithm for clinical decision making in acute pancreatitis. AIP: Acute interstitial pancreatitis; NP: Necrotizing pancreatitis; CRP: C reactive protein; CECT: Contrast-enhanced computer tomography; LDH: Lactate dehydrogenase; ERC: Endoscopic retrograde cholangiography; ES: Endoscopic sphincterotomy; CE: Laparoscopic cholecystectomy; FNP: Fine needle procedure; MOF: Multiorgan failure syndrome.

Table 5 Severe acute pancreatitis: Clinical systems to predict prognosis

	Cut-off	Time	Reference
Ranson	> 2 points	> 48 h	SGO 1974[71]
Apache Ⅱ	> 9 points	daily	Br J Surg 1990[65]
Balthazar	C, D, E	> first week	Radiology 1990[69]
Marshall score	> 3 points	72 h	Crit Car Med 1995[45]
MOF/Goris	> 1 point	48 h	Arch Surg 1985[46]
SOFA	> 4 points	48 h/d	Crit Car Med 1996[41]

MOF: Multiorgan failure; SOFA: Sepsis-related organ failure assessment.

of pancreatitis. ERCP, endoscopic sphincterotomy and stone removal are applied after subsidence of clinical signs of AP. In severe biliary pancreatitis, early sphincterotomy and stone extraction are beneficial when common bile duct stone has been diagnosed to be associated with SAP. Early endoscopic extraction of common bile duct stones brings about disappearance of cholestasis and decompression of the pancreatic main duct. A significant reduction of biliary and systemic morbidity has been objectified by two randomized controlled trials (RCTs)[74-76]. However, ERCP and sphincterotomy in SAP increases the risk of an additional pancreatitic trauma in up to 10% of patients and may increase the risk of additional cholangitis episodes during the course of SAP.

Antibiotic prophylaxis turned out to be not very effective in regard to avoidance or reduction of infection of necrosis and associated systemic complications[77]. Two randomized

Table 6 Early prediction of infected necrosis, infected necrosis + MODS and death using biochemical parameters

	Cut-off	Sensitivity (%)	Specificity (%)	Accuracy (%)
Prediction of infected necrosis				
PCT	⩾ 1.4 ng/mL	75	68	69[a]
CRP	⩾ 400 mg/L	29	92	76
Prediction of infected necrosis and MODS				
PCT	⩾ 3.8 ng/mL	80	93	92[a]
CRP	⩾ 410 mg/L	35	93	87
Prediction of death				
PCT	⩾ 3.8 ng/mL	82	88	88[a]
Prediction of IN and MODS or death				
PCT	⩾ 3.8 ng/mL	76	94	92[a]
CRP	⩾ 400 mg/L	35	92	84

PCT: Randomized controlled trial; CRP: C reactive protein; MODS: Multiorgan dysfunction syndrome; IN: Infected necrosis. Receiver operating curve-analysis based on d 3 and 4 onset of symptoms, [a]P < 0.05-0.004, Rau, Annals of Surgery 2007[72].

Table 7 Severe acute pancreatitis-antibiotic prophylaxis is inefficient in severe acute pancreatitis; results of two randomized controlled double-blind multicentric trials

	Isenmann[79]		Dellinger[80]	
	2004	*P* value	2005	*P* value
Patients (*n*)	114		100	
Treatment (*n*)[1]	48		40	
Placebo (*n*)	41		40	
Infection of necrosis				
Treatment	12%[1]	NS	23%[2]	NS
Placebo	14%[1]		15%[2]	
Need for surgery				
Treatment	17%[1]	NS	23%[2]	NS
Placebo	11%[1]		24%[2]	
Hospital mortality				
Treatment	12%[1]	NS	20%[2]	NS
Placebo	9%[1]		18%[2]	

[1]Statistical comparison of treatment and placebo group data 2004; [2]Data 2005.

double blinded prospective controlled multi-center trials proved antibiotic prophylaxis ineffective in regard to reduction of infection of necrosis and hospital mortality[78,79] (Table 7). But patients with pulmonary infection and who show a positive blood culture associated with signs of sepsis should be treated with antibiotics. Enteral feeding (EN) in SAP reduces significantly the infection rate of necrosis and lowers the need for surgical interventions[80-84]. However, hospital mortality and non-infectious complications are not altered by enteral feeding compared to parenteral nutrition (Table 8). The beneficial effect of EN may be more pronounced if it is instituted early[85].

Non-surgical ICU-management is successful in most patients with AP who have sterile pancreatic necroses and who do not develop organ failure (Table 9). Patients having pancreatic necroses and who are fine needle procedure (FNP)-positive but do not show clinical signs of sepsis, do not need surgical intervention[86-88].

Interventional treatment of infected necrosis

Surgical debridement has been documented to be

Table 8 Severe acute pancreatitis-enteral feeding reduces infection in the need for surgical intervention

Benefits of enteral nutrition	Lower infections ($P = 0.004$)
	Reduced surgical interventions ($P = 0.05$)
	Reduced LHS-2.9 d ($P < 0.001$)
Differences	Hospital mortality ($P = 0.3$)
	Non-infectious complications ($P = 0.16$)

LHS: Length of hospital stay. Enteral feeding *vs* parenteral nutrition, results of 6 RCTs, meta-analysis of 263 patients[81-86].

Table 9 Severe acute pancreatitis-surgical and non-surgical treatment: Ulm Experience: 1568 patients[1] *n* (%)

	Patients	Conservative	Surgery/Intervention
Interstitial-oedematous	1071 (68.3)	1056 (98.6)	15 (1.4)[2]
Necrotizing pancreatitis	359 (22.9)	95 (26.5)	264 (73.5)
Sterile necrosis	227	85 (37.5)	142 (62.5)
Infected necrosis	132	10 (7.6)	122 (92.4)
Pancreatic abscess	42 (2.7)	3 (7.1)	39 (92.9)
Postacute pseudocyst	96 (6.1)	22 (22.9)	74 (77.1)

[1]5/1982-12/1999 Department of General Surgery, University of Ulm, Germany. [2]Biliary tract surgery not included.

effective for patients with proven infected necrosis and progressive clinical sepsis. Patients with SAP who develop a surgical acute abdomen during the course of ICU treatment need emergency surgery to avoid development of abdominal compartment syndrome[57] or consequences of intestinal perforations. Patients with extended sterile necrosis (> 50% of the pancreas) are at high risk for infected necrosis with the consequence of progressive MODS. These patients are candidates for surgical and interventional measures after their clinical signs show non-response to maximum intensive care treatment. Patients with infected necrosis are managed by surgical and interventional treatment modalities (Table 10).

A variety of surgical treatment modalities are currently in use. The advantages of minimally invasive interventional debridement, whether performed by laparoscopic techniques or by a retroperitoneal approach, are up to now not based on results of controlled clinical trials. By use of minimal invasive techniques for infected necrosis in the late course of disease, the morbidity remains high for several days. Two to 7 reoperations with lavage are necessary to interrupt systemic complications of the local inflammatory process[95-102] (Table 11). An open surgical debridement combined with continuous short-term lavage of the lesser sack interrupts clinical sepsis in patients suffering from extended necrosis with infection accompanied by multi-system organ failure. The early treatment related morbidity is much lower in patients treated with open surgery than after first pass of minimal invasive debridement. The frequency of reoperation is between 25% and 40% and up to 100% in patients after minimal access intervention. Hospital mortality in high volume centers is below 20% after open necrosectomy plus bursa lavage and after minimal invasive surgical approach as well.

Table 10 Results of open surgical debridement of necrotizing pancreatitis using surgical debridement and local bursa lavage

	Complication			Hospital mortality
	n	Postop, *n* (%)	Reop, *n* (%)	*n* (%)
Pederzoli 1990[90]	191	55 (29)	34 (18)	40 (21)
Beger 1999[92]	221	122 (55)	93 (42)	46 (21)
Mai 2000[61]	27	10 (37)	6 (22)	5 (18)
Hungness 2002[93]	26		4 (15.4)	6 (23)
Farkas 2006[94]	220	43%	48 (22)	17 (7.7)
Howard 2007[95]	102	83 (81)	69 (68)	12 (11.8)
1990-2007	787	43%	29.60%	14.70%

Table 11 Results of minimal invasive interventional treatment of necrotizing pancreatitis: Minimal invasive debridement + local lavage

	n	Infect. necrosis (%)	Apache II	Time O-S	Early morbidity (%)	OP/ pts	Hospital mortality (%)
Freeny 1998[96]	34	100	-				20
Goiuzi 1999[97]	32	81	26				15
Carter 2000[98]	10	90		24 d	10	3	20
Horvath 2001[99]	6	100			33		0
Castellanos 2002[100]	15	100			40		27
Connor 2003[101]	24	58	8		88	4	25
Zhou 2003[102]	12	58		72/102			0
Connor 2005[103]	47	81	9	28 d	92	3	19
1998-2006	156 pts	83			70.6		18.3

OP: Operation per patient; O: Onset of disease; S: Surgery.

REFERENCES

1 **Dugernier TL**, Laterre PF, Wittebole X, Roeseler J, Latinne D, Reynaert MS, Pugin J. Compartmentalization of the inflammatory response during acute pancreatitis: correlation with local and systemic complications. *Am J Respir Crit Care Med* 2003; **168**: 148-157

2 **Mayer J**, Rau B, Gansauge F, Beger HG. Inflammatory mediators in human acute pancreatitis: clinical and pathophysiological implications. *Gut* 2000; **47**: 546-552

3 **Makhija R**, Kingsnorth AN. Cytokine storm in acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2002; **9**: 401-410

4 **Lipsett PA**. Serum cytokines, proteins, and receptors in acute pancreatitis: mediators, markers, or more of the same? *Crit Care Med* 2001; **29**: 1642-1644

5 **Pezzilli R**, Maldini M, Morselli-Labate AM, Barakat B, Romboli E, Beltrandi E, Migliori M, Tomassetti P, Corinaldesi R. Early activation of peripheral lymphocytes in human acute pancreatitis. *J Clin Gastroenterol* 2003; **36**: 360-363

6 **Poch B**, Gansauge F, Rau B, Wittel U, Gansauge S, Nüssler AK, Schoenberg M, Beger HG. The role of polymorphonuclear leukocytes and oxygen-derived free radicals in experimental acute pancreatitis: mediators of local destruction and activators of inflammation. *FEBS Lett* 1999; **461**: 268-272

7 **Sakai Y**, Masamune A, Satoh A, Nishihira J, Yamagiwa T, Shimosegawa T. Macrophage migration inhibitory factor is a critical mediator of severe acute pancreatitis. *Gastroenterology* 2003; **124**: 725-736

8 **Guzman EA**, Rudnicki M. Intricacies of host response in acute pancreatitis. *J Am Coll Surg* 2006; **202**: 509-519

9 **Deitch EA**, Xu DZ, Qi L, Berg RD. Bacterial translocation from the gut impairs systemic immunity. *Surgery* 1991; **109**: 269-276

10 **Bonham MJ**, Abu-Zidan FM, Simovic MO, Windsor JA. Gastric intramucosal pH predicts death in severe acute pancreatitis. *Br J Surg* 1997; **84**: 1670-1674

11 **Ammori BJ**. Role of the gut in the course of severe acute pancreatitis. *Pancreas* 2003; **26**: 122-129
12 **Juvonen PO**, Tenhunen JJ, Heino AA, Merasto M, Paajanen HE, Alhava EM, Takala JA. Splanchnic tissue perfusion in acute experimental pancreatitis. *Scand J Gastroenterol* 1999; **34**: 308-314
13 **Holland J**, Carey M, Hughes N, Sweeney K, Byrne PJ, Healy M, Ravi N, Reynolds JV. Intraoperative splanchnic hypoperfusion, increased intestinal permeability, down-regulation of monocyte class II major histocompatibility complex expression, exaggerated acute phase response, and sepsis. *Am J Surg* 2005; **190**: 393-400
14 **Ammori BJ**, Leeder PC, King RF, Barclay GR, Martin IG, Larvin M, McMahon MJ. Early increase in intestinal permeability in patients with severe acute pancreatitis: correlation with endotoxemia, organ failure, and mortality. *J Gastrointest Surg* 1999; **3**: 252-262
15 **Wang XD**, Wang Q, Andersson R, Ihse I. Alterations in intestinal function in acute pancreatitis in an experimental model. *Br J Surg* 1996; **83**: 1537-1543
16 **Soong CV**, Halliday MI, Barclay GR, Hood JM, Rowlands BJ, Barros D'Sa AA. Intramucosal acidosis and systemic host responses in abdominal aortic aneurysm surgery. *Crit Care Med* 1997; **25**: 1472-1479
17 **Haglund U**, Lundgren O. Intestinal ischemia and shock factors. *Fed Proc* 1978; **37**: 2729-2733
18 **Horton JW**, Walker PB. Oxygen radicals, lipid peroxidation, and permeability changes after intestinal ischemia and reperfusion. *J Appl Physiol* 1993; **74**: 1515-1520
19 **Swank GM**, Deitch EA. Role of the gut in multiple organ failure: bacterial translocation and permeability changes. *World J Surg* 1996; **20**: 411-417
20 **Norman J**, Franz M, Messina J, Riker A, Fabri PJ, Rosemurgy AS, Gower WR. Interleukin-1 receptor antagonist decreases severity of experimental acute pancreatitis. *Surgery* 1995; **117**: 648-655
21 **de Beaux AC**, Goldie AS, Ross JA, Carter DC, Fearon KC. Serum concentrations of inflammatory mediators related to organ failure in patients with acute pancreatitis. *Br J Surg* 1996; **83**: 349-353
22 **Guice KS**, Oldham KT, Caty MG, Johnson KJ, Ward PA. Neutrophil-dependent, oxygen-radical mediated lung injury associated with acute pancreatitis. *Ann Surg* 1989; **210**: 740-747
23 **Exley AR**, Leese T, Holliday MP, Swann RA, Cohen J. Endotoxaemia and serum tumour necrosis factor as prognostic markers in severe acute pancreatitis. *Gut* 1992; **33**: 1126-1128
24 **Windsor JA**, Fearon KC, Ross JA, Barclay GR, Smyth E, Poxton I, Garden OJ, Carter DC. Role of serum endotoxin and antiendotoxin core antibody levels in predicting the development of multiple organ failure in acute pancreatitis. *Br J Surg* 1993; **80**: 1042-1046
25 **Steffen EK**, Berg RD. Relationship between cecal population levels of indigenous bacteria and translocation to the mesenteric lymph nodes. *Infect Immun* 1983; **39**: 1252-1259
26 **Koh IH**, Montero EF, Keller R, Silva MH, Goldenberg S, Silva RM. Can bacterial translocation to the mesenteric lymph node be correlated with systemic infection? *Transplant Proc* 1996; **28**: 2673
27 **Beger HG**, Bittner R, Block S, Büchler M. Bacterial contamination of pancreatic necrosis. A prospective clinical study. *Gastroenterology* 1986; **91**: 433-438
28 **Banks RE**, Evans SW, Alexander D, Van Leuven F, Whicher JT, McMahon MJ. Alpha 2 macroglobulin state in acute pancreatitis. Raised values of alpha 2 macroglobulin-protease complexes in severe and mild attacks. *Gut* 1991; **32**: 430-434
29 **Mentula P**, Kylänpää ML, Kemppainen E, Jansson SE, Sarna S, Puolakkainen P, Haapiainen R, Repo H. Plasma anti-inflammatory cytokines and monocyte human leucocyte antigen-DR expression in patients with acute pancreatitis. *Scand J Gastroenterol* 2004; **39**: 178-187
30 **Kylänpää-Bäck ML**, Takala A, Kemppainen E, Puolakkainen P, Kautiainen H, Jansson SE, Haapiainen R, Repo H. Cellular markers of systemic inflammation and immune suppression in patients with organ failure due to severe acute pancreatitis. *Scand J Gastroenterol* 2001; **36**: 1100-1107
31 **Bhatnagar A**, Wig JD, Majumdar S. Immunological findings in acute and chronic pancreatitis. *ANZ J Surg* 2003; **73**: 59-64
32 **Mentula P**, Kylänpää-Bäck ML, Kemppainen E, Takala A, Jansson SE, Kautiainen H, Puolakkainen P, Haapiainen R, Repo H. Decreased HLA (human leucocyte antigen)-DR expression on peripheral blood monocytes predicts the development of organ failure in patients with acute pancreatitis. *Clin Sci* (Lond) 2003; **105**: 409-417
33 **Widdison AL**, Cunningham S. Immune function early in acute pancreatitis. *Br J Surg* 1996; **83**: 633-636
34 **Sweeney KJ**, Kell MR, Coates C, Murphy T, Reynolds JV. Serum antigen(s) drive the proinflammatory T cell response in acute pancreatitis. *Br J Surg* 2003; **90**: 313-319
35 **Larvin M**, Alexander DJ, Switala SF, McMahon MJ. Impaired mononuclear phagocyte function in patients with severe acute pancreatitis: evidence from studies of plasma clearance of trypsin and monocyte phagocytosis. *Dig Dis Sci* 1993; **38**: 18-27
36 **Granger J**, Remick D. Acute pancreatitis: models, markers, and mediators. *Shock* 2005; **24** Suppl 1: 45-51
37 **Bradley EL**. A clinically based classification system for acute pancreatitis. *Ann Chir* 1993; **47**: 537-541
38 **Fedorak IJ**, Ko TC, Djuricin G, McMahon M, Thompson K, Prinz RA. Secondary pancreatic infections: are they distinct clinical entities? *Surgery* 1992; **112**: 824-830; discussion 830-831
39 **Bittner R**, Block S, Büchler M, Beger HG. Pancreatic abscess and infected pancreatic necrosis. Different local septic complications in acute pancreatitis. *Dig Dis Sci* 1987; **32**: 1082-1087
40 **Steinberg W**, Tenner S. Acute pancreatitis. *N Engl J Med* 1994; **330**: 1198-1210
41 **Vincent JL**, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, Reinhart CK, Suter PM, Thijs LG. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 1996; **22**: 707-710
42 **Eckerwall G**, Olin H, Andersson B, Andersson R. Fluid resuscitation and nutritional support during severe acute pancreatitis in the past: what have we learned and how can we do better? *Clin Nutr* 2006; **25**: 497-504
43 **Baillargeon JD**, Orav J, Ramagopal V, Tenner SM, Banks PA. Hemoconcentration as an early risk factor for necrotizing pancreatitis. *Am J Gastroenterol* 1998; **93**: 2130-2134
44 **Brown A**, Baillargeon JD, Hughes MD, Banks PA. Can fluid resuscitation prevent pancreatic necrosis in severe acute pancreatitis? *Pancreatology* 2002; **2**: 104-107
45 **Marshall JC**, Christou NV, Meakins JL. The gastrointestinal tract. The "undrained abscess" of multiple organ failure. *Ann Surg* 1993; **218**: 111-119
46 **Goris RJ**, te Boekhorst TP, Nuytinck JK, Gimbrère JS. Multiple-organ failure. Generalized autodestructive inflammation? *Arch Surg* 1985; **120**: 1109-1115
47 **Traverso LW**, Kozarek RA. Interventional management of peripancreatic fluid collections. *Surg Clin North Am* 1999; **79**: 745-757, viii-ix
48 **Isenmann R**, Rau B, Beger HG. Early severe acute pancreatitis: characteristics of a new subgroup. *Pancreas* 2001; **22**: 274-278
49 **Tao HQ**, Zhang JX, Zou SC. Clinical characteristics and management of patients with early acute severe pancreatitis: experience from a medical center in China. *World J Gastroenterol* 2004; **10**: 919-921
50 **Rau BM**, Bothe A, Kron M, Beger HG. Role of early multisystem organ failure as major risk factor for pancreatic infections and death in severe acute pancreatitis. *Clin Gastroenterol Hepatol* 2006; **4**: 1053-1061
51 **McKay CJ**, Evans S, Sinclair M, Carter CR, Imrie CW. High early mortality rate from acute pancreatitis in Scotland, 1984-1995. *Br J Surg* 1999; **86**: 1302-1305
52 **Renner IG**, Savage WT, Pantoja JL, Renner VJ. Death due to acute pancreatitis. A retrospective analysis of 405 autopsy cases. *Dig Dis Sci* 1985; **30**: 1005-1018

53 **Wilson C**, Imrie CW, Carter DC. Fatal acute pancreatitis. *Gut* 1988; **29**: 782-788
54 **Block S**, Maier W, Bittner R, Büchler M, Malfertheiner P, Beger HG. Identification of pancreas necrosis in severe acute pancreatitis: imaging procedures versus clinical staging. *Gut* 1986; **27**: 1035-1042
55 **Beger HG**, Büchler MW. Decision-making in surgical treatment of acute pancreatitis: operative or consecutive management of necrotizin pancreatitis. *Theor Surg* 1986; **1**: 61
56 **Beger HG**, Krautzberger W, Bittner R, Block S. Results of surgical treatment of necrotizing pancreatitis. *World J Surg* 1985; **9**: 972-979
57 **Rau B**, Pralle U, Uhl W, Schoenberg MH, Beger HG. Management of sterile necrosis in instances of severe acute pancreatitis. *J Am Coll Surg* 1995; **181**: 279-288
58 **Karimgani I**, Porter KA, Langevin RE, Banks PA. Prognostic factors in sterile pancreatic necrosis. *Gastroenterology* 1992; **103**: 1636-1640
59 **Takeda K**, Matsuno S, Sunamura M, Kobari M. Surgical aspects and management of acute necrotizing pancreatitis: recent results of a cooperative national survey in Japan. *Pancreas* 1998; **16**: 316-322
60 **Rau B**, Bothe A, Beger HG. Surgical treatment of necrotizing pancreatitis by necrosectomy and closed lavage: changing patient characteristics and outcome in a 19-year, single-center series. *Surgery* 2005; **138**: 28-39
61 **Mai G**, Uhl W, Muller CH, Büchler MW. The conservative management of severe acute pancreatitis. In: Büchler M, Uhl W, Friess H, Malfertheiner P, editors. Acute Pancreatitis Novel Concepts in Biology and Therapy. Oxford: Blackwell Science Ltd., 1999: 475-485
62 **Isenmann R**, Schwarz M, Rau B, Trautmann M, Schober W, Beger HG. Characteristics of infection with Candida species in patients with necrotizing pancreatitis. *World J Surg* 2002; **26**: 372-376
63 **He YM**, Lv XS, Ai ZL, Liu ZS, Qian Q, Sun Q, Chen JW, Lei DX, Jiang CQ, Yuan YF. Prevention and therapy of fungal infection in severe acute pancreatitis: A prospective clinical study. *World J Gastroenterol* 2003; **9**: 2619-2621
64 **Rau B**, Pralle U, Mayer JM, Beger HG. Role of ultrasonographically guided fine-needle aspiration cytology in the diagnosis of infected pancreatic necrosis. *Br J Surg* 1998; **85**: 179-184
65 **Larvin M**, McMahon MJ. APACHE-II score for assessment and monitoring of acute pancreatitis. *Lancet* 1989; **2**: 201-205
66 **Uhl W**, Büchler M, Malfertheiner P, Martini M, Beger HG. PMN-elastase in comparison with CRP, antiproteases, and LDH as indicators of necrosis in human acute pancreatitis. *Pancreas* 1991; **6**: 253-259
67 **Rau B**, Steinbach G, Gansauge F, Mayer JM, Grünert A, Beger HG. The potential role of procalcitonin and interleukin 8 in the prediction of infected necrosis in acute pancreatitis. *Gut* 1997; **41**: 832-840
68 **Balthazar EJ**, Robinson DL, Megibow AJ, Ranson JH. Acute pancreatitis: value of CT in establishing prognosis. *Radiology* 1990; **174**: 331-336
69 **Rau B**, Schilling MK, Beger HG. Laboratory markers of severe acute pancreatitis. *Dig Dis* 2004; **22**: 247-257
70 **Ranson JH**, Rifkind KM, Roses DF, Fink SD, Eng K, Localio SA. Objective early identification of severe acute pancreatitis. *Am J Gastroenterol* 1974; **61**: 443-451
71 **Rau BM**, Kemppainen EA, Gumbs AA, Büchler MW, Wegscheider K, Bassi C, Puolakkainen PA, Beger HG. Early assessment of pancreatic infections and overall prognosis in severe acute pancreatitis by procalcitonin (PCT): a prospective international multicenter study. *Ann Surg* 2007; **245**: 745-754
72 **Tenner S**. Initial management of acute pancreatitis: critical issues during the first 72 hours. *Am J Gastroenterol* 2004; **99**: 2489-2494
73 **Ruokonen E**, Uusaro A, Alhava E, Takala J. The effect of dobutamine infusion on splanchnic blood flow and oxygen transport in patients with acute pancreatitis. *Intensive Care Med* 1997; **23**: 732-737
74 **Neoptolemos JP**, Carr-Locke DL, London NJ, Bailey IA, James D, Fossard DP. Controlled trial of urgent endoscopic retrograde cholangiopancreatography and endoscopic sphincterotomy versus conservative treatment for acute pancreatitis due to gallstones. *Lancet* 1988; **2**: 979-983
75 **Fan ST**, Lai EC, Mok FP, Lo CM, Zheng SS, Wong J. Early treatment of acute biliary pancreatitis by endoscopic papillotomy. *N Engl J Med* 1993; **328**: 228-232
76 **Fölsch UR**, Nitsche R, Lüdtke R, Hilgers RA, Creutzfeldt W. Early ERCP and papillotomy compared with conservative treatment for acute biliary pancreatitis. The German Study Group on Acute Biliary Pancreatitis. *N Engl J Med* 1997; **336**: 237-242
77 **de Vries AC**, Besselink, MG, Buskens E, Ridwan BU, Schipper M, van Erpecum KJ, Gooszen HG. Randomized controlled trials of antibiotic prophylaxis in severe acute pancreatitis: relationship between methodological quality and outcome. *Pancreatology* 2007 (in press)
78 **Isenmann R**, Rünzi M, Kron M, Kahl S, Kraus D, Jung N, Maier L, Malfertheiner P, Goebell H, Beger HG. Prophylactic antibiotic treatment in patients with predicted severe acute pancreatitis: a placebo-controlled, double-blind trial. *Gastroenterology* 2004; **126**: 997-1004
79 **Dellinger P**. *Antibiotic prophylaxis in severe acute pancreatitis*. Annals of Surgery 2007; In press
80 **Abou-Assi S**, Craig K, O'Keefe SJ. Hypocaloric jejunal feeding is better than total parenteral nutrition in acute pancreatitis: results of a randomized comparative study. *Am J Gastroenterol* 2002; **97**: 2255-2262
81 **Oláh A**, Pardavi G, Belágyi T, Nagy A, Issekutz A, Mohamed GE. Early nasojejunal feeding in acute pancreatitis is associated with a lower complication rate. *Nutrition* 2002; **18**: 259-262
82 **McClave SA**, Greene LM, Snider HL, Makk LJ, Cheadle WG, Owens NA, Dukes LG, Goldsmith LJ. Comparison of the safety of early enteral vs parenteral nutrition in mild acute pancreatitis. *JPEN J Parenter Enteral Nutr* 1997; **21**: 14-20
83 **Kalfarentzos F**, Kehagias J, Mead N, Kokkinis K, Gogos CA. Enteral nutrition is superior to parenteral nutrition in severe acute pancreatitis: results of a randomized prospective trial. *Br J Surg* 1997; **84**: 1665-1669
84 **Gupta R**, Patel K, Calder PC, Yaqoob P, Primrose JN, Johnson CD. A randomised clinical trial to assess the effect of total enteral and total parenteral nutritional support on metabolic, inflammatory and oxidative markers in patients with predicted severe acute pancreatitis (APACHE II > or =6). *Pancreatology* 2003; **3**: 406-413
85 **Windsor AC**, Kanwar S, Li AG, Barnes E, Guthrie JA, Spark JI, Welsh F, Guillou PJ, Reynolds JV. Compared with parenteral nutrition, enteral feeding attenuates the acute phase response and improves disease severity in acute pancreatitis. *Gut* 1998; **42**: 431-435
86 **Adler DG**, Chari ST, Dahl TJ, Farnell MB, Pearson RK. Conservative management of infected necrosis complicating severe acute pancreatitis. *Am J Gastroenterol* 2003; **98**: 98-103
87 **Manes G**, Uomo I, Menchise A, Rabitti PG, Ferrara EC, Uomo G. Timing of antibiotic prophylaxis in acute pancreatitis: a controlled randomized study with meropenem. *Am J Gastroenterol* 2006; **101**: 1348-1353
88 **Runzi M**, Niebel W, Goebell H, Gerken G, Layer P. Severe acute pancreatitis: nonsurgical treatment of infected necroses. *Pancreas* 2005; **30**: 195-199
89 **Pederzoli P**, Bassi C, Vesentini S, Girelli R, Cavallini G, Falconi M, Nifosi F, Riela A, Dagradi A. Retroperitoneal and peritoneal drainage and lavage in the treatment of severe necrotizing pancreatitis. *Surg Gynecol Obstet* 1990; **170**: 197-203
90 **Farkas G**, Márton J, Mándi Y, Szederkényi E. Surgical strategy and management of infected pancreatic necrosis. *Br J Surg* 1996; **83**: 930-933
91 **Beger HG**. Surgical management of necrotizing pancreatitis. *Surg Clin North Am* 1989; **69**: 529-549
92 **Hungness ES**, Robb BW, Seeskin C, Hasselgren PO, Luchette FA. Early debridement for necrotizing pancreatitis: is it worthwhile? *J*

Am Coll Surg 2002; **194**: 740-744; discussion 744-745
93 **Farkas G**, Márton J, Mándi Y, Leindler L. Surgical management and complex treatment of infected pancreatic necrosis: 18-year experience at a single center. *J Gastrointest Surg* 2006; **10**: 278-285
94 **Howard TJ**, Patel JB, Zyromski N, Sandrasegaran K, Yu J, Nakeeb A, Pitt HA, Lillemoe KD. Declining morbidity and mortality rates in the surgical management of pancreatic necrosis. *J Gastrointest Surg* 2007; **11**: 43-49
95 **Freeny PC**, Hauptmann E, Althaus SJ, Traverso LW, Sinanan M. Percutaneous CT-guided catheter drainage of infected acute necrotizing pancreatitis: techniques and results. *AJR Am J Roentgenol* 1998; **170**: 969-975
96 **Gouzi JL**, Bloom E, Julio C, Labbé F, Sans N, el Rassi Z, Carrère N, Pradère B. Percutaneous drainage of infected pancreatic necrosis: an alternative to surgery. *Chirurgie* 1999; **124**: 31-37
97 **Carter CR**, McKay CJ, Imrie CW. Percutaneous necrosectomy and sinus tract endoscopy in the management of infected pancreatic necrosis: an initial experience. *Ann Surg* 2000; **232**: 175-180
98 **Horvath KD**, Kao LS, Wherry KL, Pellegrini CA, Sinanan MN. A technique for laparoscopic-assisted percutaneous drainage of infected pancreatic necrosis and pancreatic abscess. *Surg Endosc* 2001; **15**: 1221-1225
99 **Castellanos G**, Piñero A, Serrano A, Llamas C, Fuster M, Fernandez JA, Parrilla P. Translumbar retroperitoneal endoscopy: an alternative in the follow-up and management of drained infected pancreatic necrosis. *Arch Surg* 2005; **140**: 952-955
100 **Connor S**, Ghaneh P, Raraty M, Rosso E, Hartley MN, Garvey C, Hughes M, McWilliams R, Evans J, Rowlands P, Sutton R, Neoptolemos JP. Increasing age and APACHE II scores are the main determinants of outcome from pancreatic necrosectomy. *Br J Surg* 2003; **90**: 1542-1548
101 **Zhou ZG**, Zheng YC, Shu Y, Hu WM, Tian BL, Li QS, Zhang ZD. Laparoscopic management of severe acute pancreatitis. *Pancreas* 2003; **27**: e46-e50
102 **Connor S**, Raraty MG, Howes N, Evans J, Ghaneh P, Sutton R, Neoptolemos JP. Surgery in the treatment of acute pancreatitis--minimal access pancreatic necrosectomy. *Scand J Surg* 2005; **94**: 135-142

S- Editor Zhu LH **L- Editor** Zhu LH **E- Editor** Wang HF

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5052-5059
World Journal of Gastroenterology ISSN 1007-9327

REVIEW

Surgical management of polycystic liver disease

Robert T Russell, C Wright Pinson

Robert T Russell, C Wright Pinson, Vanderbilt University Medical Center, Department of Hepatobiliary Surgery and Liver Transplantation, 1301 22nd Avenue South, Nashville, TN 37232-5545, United States
Supported by an educational grant from Novartis Pharmaceuticals
Correspondence to: C Wright Pinson, MD, MBA, Vanderbilt University Medical Center, Department of Hepatobiliary Surgery and Liver Transplantation, 1301 22nd Avenue South, Nashville, TN 37232-5545, United States. wright.pinson@vanderbilt.edu
Telephone: +1-615-3439324 Fax: +1-615-3436478
Received: July 13, 2007 Revised: August 2, 2007

Abstract

Adult polycystic liver disease (PCLD) is an autosomal dominant condition commonly associated with autosomal dominant polycystic kidney disease (ADPKD). However in the last decade, it has been recognized that there is a distinct form of autosomal dominant PCLD that arises without concomitant ADPKD. Early knowledge of the pathogenesis was gained from the study of hepatic cysts in patients with ADPKD. Bile duct overgrowth after embryogenesis results in cystic hepatic dilatations that are known as biliary microhamartomas or von Meyenburg complexes. Further dilatation arises from cellular proliferation and fluid secretion into these cysts. There is a variable, broad spectrum of manifestations of PCLD. Although PCLD is most often asymptomatic, massive hepatomegaly can lead to disabling symptoms of abdominal pain, early satiety, persistent nausea, dyspnea, ascites, biliary obstruction, and lower body edema. Complications of PCLD include cyst rupture and cyst infection. Also, there are associated medical problems, especially intracranial aneurysms and valvular heart disease, which clinicians need to be aware of and evaluate in patients with PCLD. In asymptomatic patients, no treatment is indicated for PCLD. In the symptomatic patient, surgical therapy is the mainstay of treatment tailored to the extent of disease for each patient. Management options include cyst aspiration and sclerosis, open or laparoscopic fenestration, liver resection with fenestration, and liver transplantation. The surgical literature discussing treatment of PCLD, including techniques, outcomes, and complication rates, are summarized in this review.

Key words: Polycystic liver disease; Fenestration; Laparoscopy; Liver resection; Liver transplantation

Russell RT, Pinson CW. Surgical management of polycystic liver disease. *World J Gastroenterol* 2007; 13(38): 5052-5059

http://www.wjgnet.com/1007-9327/13/5052.asp

INTRODUCTION

Adult polycystic liver disease (PCLD) was first described in 1856 by Bristowe in association with autosomal dominant polycystic kidney disease (ADPKD)[1]. PCLD is a rare (incidence < 0.01%) dominantly inherited disorder characterized by multiple diffuse cystic lesions of the liver parenchyma. An asymptomatic enlarged liver is usually the hallmark of the disease. However with more effective treatment of renal disease, increasing numbers of patients are living long enough to experience symptoms from their associated polycystic liver disease. Significant symptoms or complications from liver involvement can occur in up to 20 percent of cases[2,3]. In symptomatic PCLD patients, surgical therapy is the mainstay of therapy including laparoscopic or open fenestration with or without hepatic resection and orthotopic liver transplantation. The surgical therapy should be tailored to the extent of disease in each patient. In this review, we will summarize the literature addressing the clinical presentation, associated medical problems, and appropriate surgical management of patients with adult polycystic liver disease.

PATHOGENESIS AND GENETIC BASIS OF POLYCYSTIC LIVER DISEASE

Although there is an isolated form of polycystic liver disease, knowledge concerning the pathogenesis of hepatic cysts was gained from the study of hepatic cysts in ADPKD. These lesions have been attributed to bile duct overgrowth after the arrest of embryogenesis and failure of the intralobar bile ducts to involute. This involutional failure results in cystic dilations that are known as biliary microhamartomas or von Meyenburg complexes (VMC)[4]. Further study of these VMC confirmed that they maintain communication with the biliary tree[5,6]. The growth of cysts in the liver is thought to arise from cell proliferation, solute and fluid secretion into the cysts, and expansion of abnormal cell matrices. Perrone and colleagues demonstrated, via culture derived epithelial cell lines, that these cysts are of biliary origin[7]. Morphological studies demonstrate that the peripheral cysts arise from biliary microhamartomas, but the centrally located cysts arise from dilatation of the peribiliary glands in the liver[8].

ADPKD is one of the most commonly inherited diseases with an incidence of 1 in 400 to 1 in 1000. It is

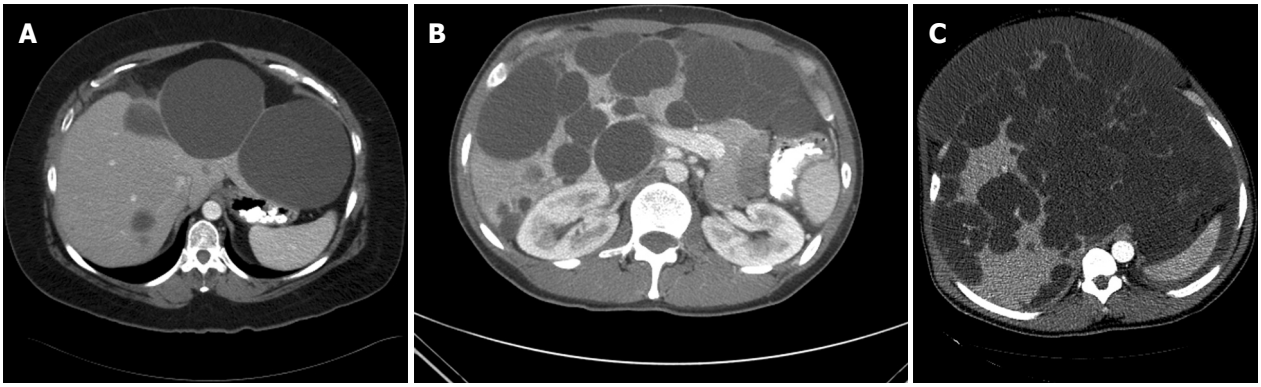


Figure 1 **A**: Type Ⅰ PCLD; **B**: Type Ⅱ PCLD; **C**: Type Ⅲ PCLD.

a cause of 8%-10% of all chronic end-stage renal failure requiring dialysis[9]. The number of ADPKD patients with hepatic involvement appears to be rising, likely due to increased life expectancy from improved renal replacement therapy and renal transplantation. Early literature suggested that 40%-50% of patients with ADPKD had polycystic liver disease[10,11], but in more recent literature, this figure has increased to 75%-90%[12]. Independent risk factors for hepatic involvement in the ADPKD include advancing patient age, female gender, and severity of renal disease. The increased prevalence in females may be due to stimulatory effects of estrogen. The reported prevalence of hepatic cysts in female patients with ADPKD ranges from 58% to 75% while the prevalence in male patients ranged from 42% to 62%[13]. Further support for the stimulatory effect of estrogen comes from studies showing an increase in liver cyst volume in pregnant women and women receiving postmenopausal estrogen therapy[14]. Finally, a correlation has been established between an increasing burden of hepatic cysts in patients as the severity of renal cystic disease increases[13].

The first suggestions of an isolated form of polycystic liver disease were made in the mid-1980's[15,16] and confirmation that there was a distinct autosomal dominant polycystic liver disease (ADPLD) occurred in the late 1990's[17,18]. ADPLD, much rarer than its PKD counterpart, has a reported incidence of less than 0.01%. ADPLD is linked to a mutation on chromosome 19 that leads to a mutated protein hepatocystin which may play a role in abnormal biliary cell proliferation and differentiation[19,20].

CLINICAL PRESENTATION

In 80% of patients, PCLD is asymptomatic[2,3] and often diagnosed incidentally during work-up of other clinical problems. In patients with long-standing disease, the most frequent symptoms are abdominal pain, early satiety, nausea and vomiting, supine shortness of breath, lower body edema, biliary obstruction, and ascites. These symptoms are usually indicative of significant hepatic enlargement or compression of adjacent structures, but rarely can be from infection or malignant transformation[21,22]. Laboratory studies including bilirubin, hepatic transaminases (AST and ALT), alkaline phosphatase, and gamma glutamyl transferase (GGT) are usually normal in asymptomatic patients. In symptomatic patients undergoing evaluation, alkaline phosphatase and GGT may be elevated in up to 47% and 70% of patients respectively[21,23-25]. AST may be elevated in up to 27% of patients and bilirubin levels may be elevated in up to 15%[21,23].

CLASSIFICATION OF APLD

Gigot and coauthors have described a detailed classification scheme for patients with polycystic liver disease based on pre-operative computed tomography (CT). This description is based on the number and size of cysts as well as the amount of residual normal liver parenchyma between the cysts[25]. Type I patients have a limited number (< 10) of large cysts with large areas of non-cystic parenchyma (Figure 1A). Patients with Type Ⅱ PCLD have diffuse involvement of liver parenchyma by medium sized cysts with remaining large areas of non-cystic parenchyma (Figure 1B). Finally, Type Ⅲ patients are characterized by massive, diffuse involvement of liver parenchyma by small and medium sized liver cysts and only a few areas of normal liver parenchyma between cysts (Figure 1C). This classification system offers a good platform for comparison of morphological disease between patients and their classification can aid us in formulating appropriate plans for therapy.

HEPATIC COMPLICATIONS OF POLYCYSTIC LIVER DISEASE

Hepatic complications from polycystic liver disease (PCLD) typically occur only in the setting of significant hepatomegaly. These cases usually present with a palpable abdominal mass, significant abdominal pain, early satiety, or dyspnea. Occasionally, severe abdominal pain will result from rupture of a hepatic cyst, hemorrhage into a cyst or if a cyst becomes infected[2,26]. Despite longstanding polycystic liver involvement, only rarely does this entity lead to hepatic insufficiency or failure. The displaced hepatic parenchyma still functions quite well.

An infected hepatic cyst is a serious, but rare, complication. These patients will usually present with fever, leukocytosis, and right upper quadrant pain. This

acute presentation should be suspected in this population and treated early to prevent progression to bacteremia, sepsis, and death. The morbidity and mortality rates of a cyst infection have been reported at 3% and 2% respectively[27]. Thus there should be early institution of antibiotics and a drainage procedure. Telenti and coauthors reviewed outcomes of patient's with infected hepatic cysts and strongly supported the use of antibiotics and a drainage procedure[28]. In their review of 14 patients with infectious complications involving hepatic cysts, seven of the fourteen patients received antibiotics alone. Of these patients, two died without having a drainage procedure, four patients underwent drainage after their symptoms persisted through antibiotic therapy, and one patient was not offered drainage due to the difficult location of the infected cyst. In the seven patients treated with combined drainage and antibiotics, one patient died from post-operative sepsis and one died from unrelated causes, while five patients had no reported morbidity from their procedures. While this literature favors antibiotic therapy along with drainage, it is often difficult to determine which cyst(s) is/are actually infected. Imaging studies can often direct us toward an area of suspicion. On CT scanning, the finding of fluid-fluid levels within cysts, cystic wall thickening, or intracystic bubbles have been associated with infection of a hepatic cyst[28,29]. Ultimately drainage of the suspected cyst provides the definitive answer whether this is from the open approach or percutaneously. In those cases where the cyst is too small or cannot be accurately identified on preoperative imaging, broad spectrum antibiotics should be instituted with narrowing of the spectrum if blood cultures implicate a specific organism[28].

Rarely, jaundice occurs as a result of cystic compression of the biliary system. This manifests as a result of the normal progression of cyst growth and compression of the common hepatic duct or common bile duct[30]. Relief of this obstruction is accomplished by surgical decompression or resection of the offending cysts.

ASSOCIATED MEDICAL CONDITIONS

Intracranial aneurysms and their association with ADPKD have been described[9,31]. There also may be increased risk for intracranial aneurysm, rupture, or dissection with ADPLD. Geevargheese and colleagues estimated the prevalence of intracranial aneurysms within this population to be 10%. Because cerebral aneurysms can be a source of morbidity and mortality in these patients, they recommended screening by magnetic resonance angiography (MRA) or computed tomographic angiography (CTA) in all patients with PCLD[32]. Also, Qian and coauthors reported an association between ADPLD and increased risk for intracranial aneurysms. In their cohort, six percent of patients with genetically confirmed ADPLD were found to have either intracranial aneurysm or dissection[33]. Although there are no definitive recommendations, these reports should encourage screening radiography and treatment prior to any surgical intervention for their PCLD.

Other associated medical conditions with ADPKD and ADPLD that clinicians should be aware of are valvular heart disease and pancreatic cysts. Classically, the valvular disease most frequently described has been mitral valve prolapse and mitral valve incompetence. In their clinical profile of ADPLD patients, Qian *et al* estimated that mitral valve prolapse occurred in up to 26% of ADPLD patients and mitral valve incompetence in up to 31% of this population[33]. This indicates cardiac evaluation in patients with ADPLD. Finally, patients with ADPKD may have asymptomatic cysts within multiple organs, including the pancreas, spleen, ovaries, and lungs. Pancreatic cysts are the most common of the extrarenal cysts with a reported incidence of 9% among ADPKD patients over 30 years[34-36].

THERAPEUTIC OPTIONS FOR APLD

The primary aims of surgical therapy for polycystic liver disease should be to significantly reduce the size of the polycystic liver without compromising liver function, and to provide long-term relief of symptoms. There is no clear consensus regarding the optimum timing of intervention and the surgical approach is based in part on the number, size, and location of the cysts. All patients should be carefully evaluated for significant symptoms and degree of disability, as well as the degree of hepatic and renal dysfunction that could affect morbidity and mortality. In addition, patients should be made aware of the risk and limitations of the surgery prior to proceeding with any surgical management.

In high risk patients and those with a large dominant cyst, percutaneous aspiration and sclerosis of cysts has been proposed as a feasible option but is associated with higher recurrence rates[37-39]. Surgical options include: laparoscopic fenestration and/or resection, open fenestration and/or resection, and liver transplantation.

FENESTRATION

Prior to the advent of laparoscopic techniques, open fenestration was the standard therapy for patients with symptomatic PCLD. The technique of fenestration was first described by Lin and coauthors in 1968[40]. This technique involves de-roofing and performing the widest possible excision of the cyst wall back to the interface of the liver parenchyma. This approach allows visualization, fenestration, and drainage of superficial and deeply seated cysts within the hepatic parenchyma and internal drainage within the peritoneal cavity. The site of fenestration must be carefully selected to avoid any bleeding or leakage of bile. Destruction of the fluid producing epithelial cyst lining, with cautery or Argon beam coagulation, may be helpful to reduce continual fluid loss from the fenestrated cysts. Patients, having type I PCLD, with superficial and large cysts of limited number are the best candidates for this procedure.

With the introduction of laparoscopy, there are increasing numbers of reports of laparoscopic fenestration of patients with PCLD[24,25,41-46]. It can be performed with similar morbidity and mortality as the open fenestration, but this approach must be utilized in the appropriate population. Patients with majority of their cysts in segments Ⅵ, Ⅶ, and often Ⅷ (when there is marked

Table 1 Open and Laparoscopic fenestration for polycystic liver disease

Reference	No. of patients	Technique (*n*)	Mortality (%)	Morbidity (%)	Mean follow-up (mo)	Rate of symptom recurrence (%)	Re-operation (%)
Lin[40]	3	Open	0	0	32	0	0
Van Erpecum[21]	9	Open	1 (11)	0	48	0	0
Turnage[47]	5	Open	1 (20)	1 (20)	10	40	0
Sanchez[67]	7	Open	0	NR	18	57	0
Farges[68]	13	Open	0	9 (69)	84	23	0
Gigot[25]	10	Open (9)	0	6 (60)	73	11	11
		Lap (1)	0	0		0	0
Koperna[41]	39	Open (34)	0	NR	75	21	21
		Lap (5)	0	NR		0	0
Morino[42]	9	Lap (9)	0	4 (44)	NR	40	NR
		Conv. (2)					
Kabbej[24]	13	Lap (13)	0	7 (54)	26	72	23
Martin[43]	13	Open (6)	0	2 (33)	96	20	20
		Lap (7)	0	2 (29)	37	71	71
Katkhouda[44]	9	Lap (9)	0	3 (33)	30	11	11
		Conv. (1)					
Fiamingo[45]	6	Lap	0	3 (50)	1-67[1]	25	0
Marks[46]	6	Lap	0	4 (67)	2-72[1]	14	0

Lap: Laparoscopic; Conv: Converted from Laparoscopic to Open; NR: Not reported. [1]Follow-up range in months.

hepatomegaly) and patients with deeply seated cysts that are difficult to visualize and fenestrate with laparoscopy may be better candidates for open fenestration. From the published series, these patients have a higher recurrence rate after laparoscopic fenestration due to the inability to adequately fenestrate all of their cysts[42,43,46]. The 13 published series describing open and/or laparoscopic fenestration are summarized in Table 1.

Koperna and colleagues reported the largest series of patients who underwent open or laparoscopic fenestration for PCLD. In their series, thirty-nine out of forty-four patients underwent a fenestration (34 open and 5 laparoscopic) for their symptomatic polycystic liver disease, while the other four underwent hepatic resection. In their experience, those patients with multiple cysts of 5 cm or greater had a higher likelihood of recurrence as compared with patients having fewer and smaller cysts (27% *vs* 13%). They performed both techniques of fenestration with no mortalities and commented that the most common morbidity was post-operative ascites. They had a mean follow-up of seventy-five months with similar overall recurrence rates between the open and laparoscopic groups (13% *vs* 11%). They concluded that in the appropriate PCLD patients, laparoscopic fenestration should replace open fenestration because it has similar rates of success along with similar morbidity and mortality rates[41]. Hepatic resection should be reserved for those patients with massive enlargement of the liver that would not benefit from simple fenestration. Combining all patients undergoing open or laparoscopic fenestration (Table 1), there was an overall high morbidity and low mortality rate of 30% and 1%, respectively. Despite an adequate fenestration, there is a moderate recurrence of symptoms and rate of re-operation (Table 1). There must be a careful evaluation of the extent of each patient's disease to determine whether fenestration alone or resection with fenestration should be recommended.

HEPATIC RESECTION WITH FENESTRATION

The combination of hepatic resection with fenestration appears to be a valuable option for those patients with symptomatic PCLD and more severe parenchymal involvement. Most of these patients are classified as Type Ⅱ or Ⅲ PCLD, based on Gigot's classification[25]. Fenestration alone in this group is rarely successful because the liver parenchyma is more rigid due to the fibrosis around the cysts and the cysts do not collapse as expected after fenestration. However, combined fenestration and resection allows for the removal of multiple segments that are grossly affected and allows for reduction in liver mass. Likewise, the large superficial and deep-seated cysts within remnant segments with more normal parenchyma can also undergo fenestration. The 10 published series are reviewed in Table 2.

The largest experience is reported by Que *et al* in a long-term follow up of 31 patients. The majority of the patients in this group had more severe parenchymal involvement (type Ⅱ and Ⅲ PCLD) necessitating resection combined with fenestration. The extent and type of liver resection depended on severity of disease with 13 patients undergoing lobectomies, 2 undergoing extended liver resections, and 16 non-anatomic liver resections. An average of 4 liver segments were resected per patient with an average weight of the resected tissue being 3.9 kg (8.6 lbs)[23]. Their mortality rate was 3% which is consistent with the other larger reported series which range from 3%-10% (Table 2). Despite a low mortality rate, the morbidity rates associated with this procedure are high and must be considered. This series reported a morbidity rate of 58%, while in other series the morbidity rates range from 20% to 100%. The most commonly reported morbidities are ascites, pleural effusions, transient biliary leaks, bleeding, and wound

Table 2 Hepatic resection with and without fenestration for polycystic liver disease

Author	No. of patients	Technique (*n*)	Mortality (%)	Morbidity (%)	Mean follow-up (mo)	Rate of symptom recurrence (%)	Re-operation (%)
Turnage[47]	3	Fen & Res	2 (67)	2 (67)	9.6	33	0
Vauthey[2]	5	Fen & Res	0	5 (100)	14	0	0
Henne-Bruns[48]	8	Fen & Res	0	3 (38)	15	50	0
Que[23]	31	Fen & Res	1 (3)	18 (58)	28	3	0
Soravia[49]	10	Fen & Res	1 (10)	2 (20)	68	33	11
Koperna[41]	5	Fen & Res	0	NR	NR	0	0
Martin[43]	9	Res	0	6 (67)	9	33	0
Vons[50]	12	Res	1 (8)	10 (83)	34	17	0
Hansman[51]	2	Res	0	0	NR	100	0
Yang[52]	7	Fen & Res	0	7 (100)	20	100	0

Fen: Fenestration; Res: Resection; NR: Not reported.

infection[2,43,47-52]. Que and coauthors had excellent results with an extremely low recurrence rate with 30 out of 31 patients remaining asymptomatic at a median follow-up of 28 mo. Importantly, they felt the extent of resection and fenestration was important for good long term outcomes. Overall, most of these patients had an improvement in their quality of life and functional status without deterioration in their hepatic or renal function[23]. Although there are high morbidity rates, resection and fenestration provides patients' with severe parenchymal involvement an opportunity for symptomatic and clinical improvement with an acceptable recurrence rate.

LIVER TRANSPLANTATION

Liver transplantation as treatment for advanced PCLD, while more accepted in recent literature, still has a limited role in management of these patients. Although a majority of PCLD patients have normal liver function, orthotopic and living donor liver transplantation have been successfully utilized in the treatment of symptomatic PCLD[53-64]. Aspiration, fenestration, or surgical resection can provide adequate palliation to those patients with large single cysts or dominant disease in one lobe, but the treatment of small, truly diffuse, cystic type PCLD may well require transplantation. Total hepatectomy and liver transplantation offers the chance of definitive treatment for this disease, but may be considered drastic, considering the absence of liver failure, the potential morbidity and mortality, and the organ shortage. In their early report of transplantation for PCLD, Starzl and colleagues described a "syndrome of lethal exhaustion" as the major indication to offer transplantation to these patients[53]. These patients often reach the end of their functional lives, have intractable pain, and have a severely diminished quality of life. Indications for transplantation include cachexia, weight loss, recurrent cyst infections, portal hypertension, and ascites. Early reports have proposed these patients not wait until end-stage complications of their PCLD become manifest before offering the option of transplantation[56,58,59]. Transplantation in those with end-stage PCLD, exhibited by severe disability, weakness, and malnutrition, has been shown to have higher infection-related mortality in early liver transplant series[56,59]. Performing earlier transplantation in appropriate candidates would seem to offer a greater chance of improved outcomes, meaningful recovery, and return to their prior functional status and quality of life. Furthermore, patients who have undergone prior more conservative therapies (aspiration, sclerosis, fenestration, or resection) may have post-surgical changes that make transplantation much more difficult[53,58,59].

The option of transplantation should be balanced against the risks of surgery, long-term immunosuppression, and the need for concurrent or subsequent renal transplantation in those with ADPKD. Thus, transplantation should be limited to those patients with Type Ⅱ/Ⅲ PCLD with diffuse, small cystic disease that would not benefit from previously described therapies. Although the first reports of transplantation for PCLD by Kwok *et al*[65] and Starzl *et al*[53] occurred in the early 1990's, eleven to reflect studies in Table 3 describing transplantation for PCLD (Table 3). Two of the largest series reported by Lange *et al* and Pirenne *et al* report the outcomes of transplantation for PCLD in 17 and 16 patients, respectively[56,59]. Lang and coauthors reported symptomatic relief in all patients following transplantation; however they did have 5 mortalities (29%) in their series. All five of these patients had severe anorexia, physical exhaustion, and evidence of malnutrition from end-stage PCLD prior to transplant and had postoperative infectious complications leading to their mortality. These deaths occurred at a mean of 41 d[56]. Pirenne and colleagues reviewed their experience of 16 patients undergoing liver transplantation for severe PCLD. They reported two mortalities (12.5%): one intra-operative death from bleeding and air emboli in a patient who had undergone previous resection, and a second late death from post-transplant lung cancer. Patient and graft survival rates were 87.5% with follow-up from 3 mo to 10 years[59]. In summary, liver transplantation offers the chance of immediate, complete, and definitive treatment in those patients with massive hepatomegaly secondary to diffuse PCLD. In these patients, fenestration and resection only offers temporary palliation, puts them at risk for potential morbidity and mortality, and jeopardizes the chances of further definitive treatment by transplantation. Several other series and their results are

Table 3 Liver Transplantation for polycystic liver disease

Author	No. of patients	Previous surgical procedure	Combined liver/Kidney Tx	Mortality	Mean follow-up (mo)
Kwok[65]	1	1	1	1	-
Starzl[53]	4	0	1	2	26
Uddin[54]	3	0	0	0	NR
Washburn[55]	5	4	1	1	38
Lang[56]	17	6	8	5	12
Swenson[58]	9	4	3	1	26
Pirenne[59]	16	4	1	2	18-120[2]
Takegoshi[60]	1[1]	0	0	0	8
Koyama[61]	1[1]	0	0	0	18
Gustafsson[63]	7	4	3	0	4
Becker[62]	17	NR	17	3	49
Ueda[64]	3[1]	NR	0	0	32
Kirchner[66]	36	NR	15	5	62

NR: Not reported. [1]Indicates living-donor transplantation, [2]Range of follow-up in months.

reviewed in Table 3.

As more patients undergo transplantation for PCLD, it is important to assess their long term outcomes, especially quality of life. Kirchner and colleagues reviewed the quality of life, *via* the SF-36 and a self-designed questionnaire, in 23 of 36 patients who underwent liver or combined liver-kidney transplantation for PCLD. Of the respondents, 91% of patients felt "much better" or "better", while only 9% felt "worse" than before. Fatigue, physical fitness, anorexia, vomiting, physical attractiveness, and interest in sex improved significantly after transplantation. Overall, patients with advanced PCLD have an improved quality of life after liver or combined liver-kidney transplantation[66].

CONCLUSION

The management of patients with PCLD continues to be challenging. In the past several decades, there have been great advances in the knowledge of the pathogenesis, genetics, and effective treatment for PCLD. Understanding this disease, potential complications, associated medical conditions, and successful treatment strategies is essential for gastroenterologists and hepatobiliary surgeons. The ability to risk-stratify these patients by severity of disease can lead to earlier interventions and attempts at prevention of massive hepatomegaly that can be so debilitating. In patients with symptomatic PCLD, invasive management strategies should be based on the degree of symptoms, the severity of associated medical conditions, and the extent of their disease. Those symptomatic patients with large cysts or limited hepatic involvement would likely benefit from laparoscopic fenestration. Adequate hepatic resection with fenestration should be favored in patients with diffuse involvement of certain areas of hepatic parenchyma with remaining large areas of non-cystic parenchyma. Finally in the patient with diffuse, small cysts, transplantation is a valid option and should be pursued as primary therapy prior to development of debilitating disease that can increase complication rates.

REFERENCES

1 **Bristowe F**. Cystic disease of the liver associated with similar disease of the kidneys. *Trans Pathol Soc Lond* 1856; **7**: 229-234
2 **Vauthey JN**, Maddern GJ, Blumgart LH. Adult polycystic disease of the liver. *Br J Surg* 1991; **78**: 524-527
3 **Grünfeld JP**, Albouze G, Jungers P, Landais P, Dana A, Droz D, Moynot A, Lafforgue B, Boursztyn E, Franco D. Liver changes and complications in adult polycystic kidney disease. *Adv Nephrol Necker Hosp* 1985; **14**: 1-20
4 **Redston MS**, Wanless IR. The hepatic von Meyenburg complex: prevalence and association with hepatic and renal cysts among 2843 autopsies corrected. *Mod Pathol* 1996; **9**: 233-237
5 **Grimm PC**, Crocker JF, Malatjalian DA, Ogborn MR. The microanatomy of the intrahepatic bile duct in polycystic disease: comparison of the cpk mouse and human. *J Exp Pathol* (Oxford) 1990; **71**: 119-131
6 **Ramos A**, Torres VE, Holley KE, Offord KP, Rakela J, Ludwig J. The liver in autosomal dominant polycystic kidney disease. Implications for pathogenesis. *Arch Pathol Lab Med* 1990; **114**: 180-184
7 **Perrone RD**, Grubman SA, Rogers LC, Lee DW, Moy E, Murray SL, Torres VE, Jefferson DM. Continuous epithelial cell lines from ADPKD liver cysts exhibit characteristics of intrahepatic biliary epithelium. *Am J Physiol* 1995; **269**: G335-G345
8 **Kida T**, Nakanuma Y, Terada T. Cystic dilatation of peribiliary glands in livers with adult polycystic disease and livers with solitary nonparasitic cysts: an autopsy study. *Hepatology* 1992; **16**: 334-340
9 **Gabow PA**. Autosomal dominant polycystic kidney disease. *N Engl J Med* 1993; **329**: 332-342
10 Feldman M. Polycystic disease of the liver. *Am J Gastroenterol* 1958; **29**: 83-86
11 **Iglesias CG**, Torres VE, Offord KP, Holley KE, Beard CM, Kurland LT. Epidemiology of adult polycystic kidney disease, Olmsted County, Minnesota: 1935-1980. *Am J Kidney Dis* 1983; **2**: 630-639
12 **D'Agata ID**, Jonas MM, Perez-Atayde AR, Guay-Woodford LM. Combined cystic disease of the liver and kidney. *Semin Liver Dis* 1994; **14**: 215-228
13 **Gabow PA**, Johnson AM, Kaehny WD, Manco-Johnson ML, Duley IT, Everson GT. Risk factors for the development of hepatic cysts in autosomal dominant polycystic kidney disease. *Hepatology* 1990; **11**: 1033-1037
14 **Sherstha R**, McKinley C, Russ P, Scherzinger A, Bronner T, Showalter R, Everson GT. Postmenopausal estrogen therapy selectively stimulates hepatic enlargement in women with autosomal dominant polycystic kidney disease. *Hepatology* 1997; **26**: 1282-1286
15 **Berrebi G**, Erickson RP, Marks BW. Autosomal dominant

polycystic liver disease: a second family. *Clin Genet* 1982; **21**: 342-347
16 **Karhunen PJ**, Tenhu M. Adult polycystic liver and kidney diseases are separate entities. *Clin Genet* 1986; **30**: 29-37
17 **Pirson Y**, Lannoy N, Peters D, Geubel A, Gigot JF, Breuning M, Verellen-Dumoulin C. Isolated polycystic liver disease as a distinct genetic disease, unlinked to polycystic kidney disease 1 and polycystic kidney disease 2. *Hepatology* 1996; **23**: 249-252
18 **Iglesias DM**, Palmitano JA, Arrizurieta E, Kornblihtt AR, Herrera M, Bernath V, Martin RS. Isolated polycystic liver disease not linked to polycystic kidney disease 1 and 2. *Dig Dis Sci* 1999; **44**: 385-388
19 **Reynolds DM**, Falk CT, Li A, King BF, Kamath PS, Huston J, Shub C, Iglesias DM, Martin RS, Pirson Y, Torres VE, Somlo S. Identification of a locus for autosomal dominant polycystic liver disease, on chromosome 19p13.2-13.1. *Am J Hum Genet* 2000; **67**: 1598-1604
20 **Drenth JP**, Tahvanainen E, te Morsche RH, Tahvanainen P, Kääriäinen H, Höckerstedt K, van de Kamp JM, Breuning MH, Jansen JB. Abnormal hepatocystin caused by truncating PRKCSH mutations leads to autosomal dominant polycystic liver disease. *Hepatology* 2004; **39**: 924-931
21 **van Erpecum KJ**, Janssens AR, Terpstra JL, Tjon A Tham RT. Highly symptomatic adult polycystic disease of the liver. A report of fifteen cases. *J Hepatol* 1987; **5**: 109-117
22 **Korobkin M**, Stephens DH, Lee JK, Stanley RJ, Fishman EK, Francis IR, Alpern MB, Rynties M. Biliary cystadenoma and cystadenocarcinoma: CT and sonographic findings. *AJR Am J Roentgenol* 1989; **153**: 507-511
23 **Que F**, Nagorney DM, Gross JB, Torres VE. Liver resection and cyst fenestration in the treatment of severe polycystic liver disease. *Gastroenterology* 1995; **108**: 487-494
24 **Kabbej M**, Sauvanet A, Chauveau D, Farges O, Belghiti J. Laparoscopic fenestration in polycystic liver disease. *Br J Surg* 1996; **83**: 1697-1701
25 **Gigot JF**, Jadoul P, Que F, Van Beers BE, Etienne J, Horsmans Y, Collard A, Geubel A, Pringot J, Kestens PJ. Adult polycystic liver disease: is fenestration the most adequate operation for long-term management? *Ann Surg* 1997; **225**: 286-294
26 **Chauveau D**, Fakhouri F, Grunfeld JP. Liver involvement in autosomal-dominant polycystic kidney disease: therapeutic dilemma. *J Am Soc Nephrol* 2000; **11**: 1767-1775
27 **Abascal J**, Moya M, Martin F. Infection of hepatic cysts in polycystic disease. *World J Surg* 1984; **8**: 424-425
28 **Telenti A**, Torres VE, Gross JB, Van Scoy RE, Brown ML, Hattery RR. Hepatic cyst infection in autosomal dominant polycystic kidney disease. *Mayo Clin Proc* 1990; **65**: 933-942
29 **Yoshida H**, Onda M, Tajiri T, Mamada Y, Taniai N, Mineta S, Hirakata A, Futami R, Arima Y, Inoue M, Hatta S, Kishimoto A. Infected hepatic cyst. *Hepatogastroenterology* 2003; **50**: 507-509
30 **Dmitrewski J**, Olliff S, Buckels JA. Obstructive jaundice associated with polycystic liver disease. *HPB Surg* 1996; **10**: 117-120
31 **Chapman AB**, Rubinstein D, Hughes R, Stears JC, Earnest MP, Johnson AM, Gabow PA, Kaehny WD. Intracranial aneurysms in autosomal dominant polycystic kidney disease. *N Engl J Med* 1992; **327**: 916-920
32 **Geevarghese SK**, Powers T, Marsh JW, Pinson CW. Screening for cerebral aneurysm in patients with polycystic liver disease. *South Med J* 1999; **92**: 1167-1170
33 **Qian Q**, Li A, King BF, Kamath PS, Lager DJ, Huston J, Shub C, Davila S, Somlo S, Torres VE. Clinical profile of autosomal dominant polycystic liver disease. *Hepatology* 2003; **37**: 164-171
34 **Torra R**, Nicolau C, Badenas C, Navarro S, Pérez L, Estivill X, Darnell A. Ultrasonographic study of pancreatic cysts in autosomal dominant polycystic kidney disease. *Clin Nephrol* 1997; **47**: 19-22
35 **Blyth H**, Ockenden BG. Polycystic disease of kidney and liver presenting in childhood. *J Med Genet* 1971; **8**: 257-284
36 **Milutinovic J**, Schabel SI, Ainsworth SK. Autosomal dominant polycystic kidney disease with liver and pancreatic involvement in early childhood. *Am J Kidney Dis* 1989; **13**: 340-344
37 **Andersson R**, Jeppsson B, Lunderquist A, Bengmark S. Alcohol sclerotherapy of non-parasitic cysts of the liver. *Br J Surg* 1989; **76**: 254-255
38 **Kairaluoma MI**, Leinonen A, Ståhlberg M, Päivänsalo M, Kiviniemi H, Siniluoto T. Percutaneous aspiration and alcohol sclerotherapy for symptomatic hepatic cysts. An alternative to surgical intervention. *Ann Surg* 1989; **210**: 208-215
39 **Tikkakoski T**, Mäkelä JT, Leinonen S, Päivänsalo M, Merikanto J, Karttunen A, Siniluoto T, Kairaluoma MI. Treatment of symptomatic congenital hepatic cysts with single-session percutaneous drainage and ethanol sclerosis: technique and outcome. *J Vasc Interv Radiol* 1996; **7**: 235-239
40 **Lin TY**, Chen CC, Wang SM. Treatment of non-parasitic cystic disease of the liver: a new approach to therapy with polycystic liver. *Ann Surg* 1968; **168**: 921-927
41 **Koperna T**, Vogl S, Satzinger U, Schulz F. Nonparasitic cysts of the liver: results and options of surgical treatment. *World J Surg* 1997; **21**: 850-854; discussion 854-855
42 **Morino M**, De Giuli M, Festa V, Garrone C. Laparoscopic management of symptomatic nonparasitic cysts of the liver. Indications and results. *Ann Surg* 1994; **219**: 157-164
43 **Martin IJ**, McKinley AJ, Currie EJ, Holmes P, Garden OJ. Tailoring the management of nonparasitic liver cysts. *Ann Surg* 1998; **228**: 167-172
44 **Katkhouda N**, Hurwitz M, Gugenheim J, Mavor E, Mason RJ, Waldrep DJ, Rivera RT, Chandra M, Campos GM, Offerman S, Trussler A, Fabiani P, Mouiel J. Laparoscopic management of benign solid and cystic lesions of the liver. *Ann Surg* 1999; **229**: 460-466
45 **Fiamingo P**, Tedeschi U, Veroux M, Cillo U, Brolese A, Da Rold A, Madia C, Zanus G, D'Amico DF. Laparoscopic treatment of simple hepatic cysts and polycystic liver disease. *Surg Endosc* 2003; **17**: 623-626
46 **Marks J**, Mouiel J, Katkhouda N, Gugenheim J, Fabiani P. Laparoscopic liver surgery. A report on 28 patients. *Surg Endosc* 1998; **12**: 331-334
47 **Turnage RH**, Eckhauser FE, Knol JA, Thompson NW. Therapeutic dilemmas in patients with symptomatic polycystic liver disease. *Am Surg* 1988; **54**: 365-372
48 **Henne-Bruns D**, Klomp HJ, Kremer B. Non-parasitic liver cysts and polycystic liver disease: results of surgical treatment. *Hepatogastroenterology* 1993; **40**: 1-5
49 **Soravia C**, Mentha G, Giostra E, Morel P, Rohner A. Surgery for adult polycystic liver disease. *Surgery* 1995; **117**: 272-275
50 **Vons C**, Chauveau D, Martinod E, Smadja C, Capron F, Grunfeld JP, Franco D. Liver resection in patients with polycystic liver disease. *Gastroenterol Clin Biol* 1998; **22**: 50-54
51 **Hansman MF**, Ryan JA, Holmes JH, Hogan S, Lee FT, Kramer D, Biehl T. Management and long-term follow-up of hepatic cysts. *Am J Surg* 2001; **181**: 404-410
52 **Yang GS**, Li QG, Lu JH, Yang N, Zhang HB, Zhou XP. Combined hepatic resection with fenestration for highly symptomatic polycystic liver disease: A report on seven patients. *World J Gastroenterol* 2004; **10**: 2598-2601
53 **Starzl TE**, Reyes J, Tzakis A, Mieles L, Todo S, Gordon R. Liver transplantation for polycystic liver disease. *Arch Surg* 1990; **125**: 575-577
54 U**ddin W**, Ramage JK, Portmann B, Wilson P, Benjamin I, Tan KC, Williams R. Hepatic venous outflow obstruction in patients with polycystic liver disease: pathogenesis and treatment. *Gut* 1995; **36**: 142-145
55 **Washburn WK**, Johnson LB, Lewis WD, Jenkins RL. Liver transplantation for adult polycystic liver disease. *Liver Transpl Surg* 1996; **2**: 17-22
56 **Lang H**, von Woellwarth J, Oldhafer KJ, Behrend M, Schlitt HJ, Nashan B, Pichlmayr R. Liver transplantation in patients with polycystic liver disease. *Transplant Proc* 1997; **29**: 2832-2833
57 **Jeyarajah DR**, Gonwa TA, Testa G, Abbasoglu O, Goldstein R, Husberg BS, Levy MF, Klintmalm GB. Liver and kidney transplantation for polycystic disease. *Transplantation* 1998; **66**: 529-532
58 **Swenson K**, Seu P, Kinkhabwala M, Maggard M, Martin P,

Goss J, Busuttil R. Liver transplantation for adult polycystic liver disease. *Hepatology* 1998; **28**: 412-415

59 **Pirenne J**, Aerts R, Yoong K, Gunson B, Koshiba T, Fourneau I, Mayer D, Buckels J, Mirza D, Roskams T, Elias E, Nevens F, Fevery J, McMaster P. Liver transplantation for polycystic liver disease. *Liver Transpl* 2001; **7**: 238-245

60 **Takegoshi K**, Tanaka K, Nomura H, Miyagi K, Taira S, Takayanagi N. Successful living donor liver transplantation for polycystic liver in a patient with autosomal-dominant polycystic kidney disease. *J Clin Gastroenterol* 2001; **33**: 229-231

61 **Koyama I**, Fuchinoue S, Urashima Y, Kato Y, Tsuji K, Kawase T, Murakami T, Tojimbara T, Nakajima I, Teraoka S. Living related liver transplantation for polycystic liver disease. *Transpl Int* 2002; **15**: 578-580

62 **Demirci G**, Becker T, Nyibata M, Lueck R, Bektas H, Lehner F, Tusch G, Strassburg C, Schwarz A, Klempnauer J, Nashan B. Results of combined and sequential liver-kidney transplantation. *Liver Transpl* 2003; **9**: 1067-1078

63 **Gustafsson BI**, Friman S, Mjornstedt L, Olausson M, Backman L. Liver transplantation for polycystic liver disease-indications and outcome. *Transplant Proc* 2003; **35**: 813-814

64 **Ueda M**, Egawa H, Oike F, Taira K, Uryuhara K, Fujimoto Y, Kozaki K, Tanaka K. Living-donor liver transplantation for polycystic liver disease. *Transplantation* 2004; **77**: 480-481

65 **Kwok MK**, Lewin KJ. Massive hepatomegaly in adult polycystic liver disease. *Am J Surg Pathol* 1988; **12**: 321-324

66 **Kirchner GI**, Rifai K, Cantz T, Nashan B, Terkamp C, Becker T, Strassburg C, Barg-Hock H, Wagner S, Lück R, Klempnauer J, Manns MP. Outcome and quality of life in patients with polycystic liver disease after liver or combined liver-kidney transplantation. *Liver Transpl* 2006; **12**: 1268-1277

67 **Sanchez H**, Gagner M, Rossi RL, Jenkins RL, Lewis WD, Munson JL, Braasch JW. Surgical management of nonparasitic cystic liver disease. *Am J Surg* 1991; **161**: 113-118; discussion 118-119

68 **Farges O**, Bismuth H. Fenestration in the management of polycystic liver disease. *World J Surg* 1995; **19**: 25-30

S- Editor Liu Y **L- Editor** Alpini GD **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5060-5064
World Journal of Gastroenterology ISSN 1007-9327

GASTRIC CANCER

Rationales for expression and altered expression of apoptotic protease activating factor-1 gene in gastric cancer

He-Ling Wang, Han Bai, Yan Li, Jun Sun, Xue-Qing Wang

He-Ling Wang, Yan Li, Jun Sun, Xue-Qing Wang, Department of Gastroenterology, Shengjing Hospital Affiliated to China Medical University, Shenyang 110004, Liaoning Province, China
Han Bai, Department of Infectious Diseases, Shengjing Hospital Affiliated to China Medical University, Shengyang 110004, Liaoning Province, China
Correspondence to: Yan Li, Department of Gastroenterology, Shengjing Hospital Affiliated to China Medical University, Shenyang110004, Liaoning Province, China. yanli0227@126.com
Telephone: +86-24-83956986 Fax: +86-24-23582697
Received: May 20, 2007 Revised: June 18, 2007

Abstract

AIM: To elucidate the relationship between apoptotic protease activating factor-1 (Apaf-1) gene and gastric cancer.

METHODS: Thirty-five postoperative cancer and adjacent normal tissue samples were collected in the present study. Expression of the Apaf-1 gene in these samples was analyzed by semi-quantitative RT-PCR. Loss of heterozygosity (LOH) was used to determine whether there was loss of Apaf-1 gene in domain of 12q22-23 in the samples. Promoter methylation of Apaf-1 gene in the samples was analyzed by methylation specific (MSP) PCR.

RESULTS: The expression of Apaf-1 mRNA in gastric cancer tissue samples was 51%. The LOH frequency of D12S346, D12S1706, D12S327, D12S1657 and D12S393 was 33%, 8%, 58%, 12% and 42%, respectively. Fifty percent LOH was found at two sites and 17% LOH at three sites. Apaf-1 mRNA expression decreased significantly in 13 cases (r_s = 0.487, P = 0.003). The rate of Apaf-1 promoter methylation was 49% in gastric cancer tissue samples and 23% in para-cancerous tissue samples. Promoter methylation occurred significantly in 16 of 18 gastric cancer tissue samples with decreased expression of Apaf-1 mRNA r_s = 0.886, $P = 10^{-6}$).

CONCLUSION: The expression of Apaf-1 gene is low in gastric cancer tissues. Methylation of Apaf-1 gene promoter and LOH in domain of 12q22-23 are the main reasons for the expression and altered expression of Apaf-1 gene.

Key words: Gastric cancer; Apaf-1 gene; Loss of heterozygosity; Methylation

Wang HL, Bai H, Li Y, Sun J, Wang XQ. Rationales for expression and altered expression of apoptotic protease activating factor-1 gene in gastric cancer. *World J Gastroenterol* 2007; 13(38): 5060-5064

http://www.wjgnet.com/1007-9327/13/5060.asp

INTRODUCTION

Formation and progression of gastric cancer are a continuous multiple-step process. Cell apoptosis is one of the main mechanisms for tumor genesis[1-3], and Apaf-1 protein is an important apoptosis factor which is expressed abnormally in a series of tumor studies[4-7]. It was recently reported that Apaf-1 gene is closely related to several cancer-inducing genes and tumor suppressor genes, such as p53 and Bcl2[8-10]. However, the expression condition of Apaf-1 gene in gastric cancer and its correlation with the genesis of gastric cancer remain unclear. This study was to study the relation between Apaf-1 gene expression and gastric cancer genesis in promoter domain.

MATERIALS AND METHODS

Materials

Thirty-five postoperative cancer tissue and adjacent normal tissue samples (> 5 cm away from the center of cancer) were collected from gastric cancer patients from January 2005 to September 2005 at No.1 and No.2 Affiliated Hospitals of China Medical University. All these patients were pathologically diagnosed according to the new TNM classification criteria of Union Internationale Contre le Cancer[11]. Of the 35 patients with progressive gastric cancer (25 males and 10 females with a mean age of 58.51 ± 12.24 years), 10 had moderately differentiated gastric cancer and 25 had poorly-differentiated gastric cancer, while 25 had lymphatic metastasis and 10 had no lymphatic metastasis. Tumor and adjacent normal tissues were isolated and maintained at -70℃. Total RNA extracting reagent Trizol (Invitrogen Co.), RT-PCR kit (TaKaRa Co.), methylating reagent (Sigma Co.) and Wizard DNA clean-up (Promega Co.) were used in the study.

Semi-quantitative RT-PCR analysis

Total RNA was extracted with Trizol reagent and cDNA

Table 1 Polymorphic sites, primers and amplification conditions (12q22-23)

Site	Repeat	Heterozygosity	Amplification length (kb)	Primer sequence	Reaction conditions
D12S327	Dinucleotide	0.8501	0.1820-0.2010	Aaa gtt tct gga tgg taa tat cg Aga gca aga cct tgt ctc aa	54℃ 40 s 72℃ 45 s
D12S1657	Dinucleotide	0.6439	0.1500-0.1600	Tcc taa aga tgg tgt gca t Aag ttc caa tgt tag tga acc	58℃ 40 s 72℃ 45 s
D12S393	Tetranucleotide	0.6410	0.2490	Att att gcc agg aca tta aac g Cct cac aca atg ttg taa ggg	58℃ 30 s 72℃ 40 s
D12S1706	Dinucleotide	0.8916	0.1190-0.1390	Cct atg att tcc cat caa gtt t Att att agg aga gcc ctg gg	57℃ 40 s 72℃ 40 s
D12S346	Dinucleotide	0.8400	0.1660	Tgc cc acct gcc tgt aac Aat gga ggg taa atg ccc g	58℃ 30 s 72℃ 40 s

was synthesized with reverse transcriptase and Oliga $(dT)_{20}$ primer. Apaf-1 primer sequence was designed with primer 3, including functional caspase recruitment domain (CARD), upstream primer (5'-TTGCTGCCCTTCTCC ATG AT-3'), downstream primer (5'-TCCCAACTGAAA CCCAATGC-3') and amplification length (334 bp). The internal reference primer was β-actin, including upstream primer (5'-GTGGGGCGCCCCAGGCACCA-3'), downstream primer (5'-CTCCTTAATGTCACGCAC GATTTC-3') and amplification length (498 bp). Both pairs of primers were added into 25 μL reaction system to react under the proper PCR reaction conditions (denatured at 94℃ for 45 s, renatured at 58℃ for 45 s and elongated at 72℃ for 60 s, for 35 cycles). RT-PCR products were isolated with 2% agarose gel, imaged through Alpha Image 2000 automatic imager and analyzed using Fluorchem V 2.0 Stand Alone software.

LOH analysis

Down-regulation of Apaf-1 gene expression was correlated with allele loss at its 5 sites (D12S327, D12S1657, D12S393, D12S1706 and D12S346). These 5 polymorphic sites were selected for LOH analysis. DNA was extracted from tumor and normal tissues using phenol/chlorine method. Primer sequences derived from genome database (GDB) and amplification conditions are listed in Table 1. Two percent agarose gel electrophoresis revealed target genes but no hybrid band in each specimen. PCR products were added into the denaturing buffer (98% formamide, 10 mmol/L EDTA, 0.025% bromophenol blue and 0.025% xylene cyanol) at a ratio of 1:1, mixed uniformly, denatured at 97℃ for 10 min and immediately placed into ice. Fifteen μL of specimens was loaded in each hole, separated through 9% non-denaturing PAGE (in a 100 V low-temperature water bath for 2-3 h) and the electrophoresis results were analyzed through silver staining.

Methylation analysis at Apaf-1 promoter domain

DNA extracted from gastric cancer and adjacent normal tissues was treated with Na_2SO_3, purified and recovered with Wizard DNA clean-up (Promega Co.) for MSP analysis. A 49-378 base zone rich in CpG at Apaf-1 cDNA promoter domain was selected to design MSP primers, including upstream primer (5'-GAGGTGTCGTAG CGGTATTC-3'), downstream primer (5'-CGAAAATTA ACGAAATAAACGTC-3'), PCR reaction conditions (denatured at 94℃ for 45 s, renatured at 58℃ for 45 s and elongated at 72℃ for 60 s, for 38 cycles) and amplification length (221 bp). Methylating primers included upstream primer (5'-ATTTGAGGT GTTGTAGTGGTATTT G-3'), downstream primer (5'-ACCTCCAAAAATTAACAA AATAAACAT-3'), PCR reaction conditions (denatured at 94℃ for 45 s, renatured at 56℃ for 45 s, elongated at 72℃ for 60 s, for 38 cycles) and amplification length (221 bp).

Statistical analysis

Statistical analyses were performed by the SPSS13.0 software package (SPSS, Inc, Chicago, IL). Continuous Data were expressed as mean ± SD. Continuous variables were compared by *t*-test when appropriate, whereas categorical variables were compared by χ^2 test or Fisher's exact test when appropriate. Spearman rank correlation was analyzed. $P < 0.05$ was considered statistically significant.

RESULTS

Gene expression

The gene expression was increased 30%-70% in cancer tissue samples with an increased difference between Apaf-1/β-actin cDNA optical density ratios in gastric cancer and adjacent normal tissue samples. In the present study, Apaf-1expression decreased in 48.57% (17/35) of gastric cancer tissue samples (including expression loss in 2 cases), paired *t* test with SPSS13.0 statistical software showed that the relative Apaf-1 content was 0.96 ± 0.40 in adjacent normal tissue samples and 0.69 ± 0.36 in cancer tissue samples, respectively ($t = 3.518$, $P < 0.01$), and Apaf-1expression was significantly higher in adjacent normal tissue samples than in cancer tissue samples. Apaf-1 mRNA expression did not increase in tumor tissue samples (Figures 1 and 2).

LOH analysis

The data from 2 fragments (i.e. genome DNA was a certain marked heterozygote in normal tissue or adjacent normal tissue samples) were sufficient for LOH analysis, but the data from one fragment were not sufficient for LOH analysis. LOH meant that there was 1 band with less genome DNA PCR amplification product or 1

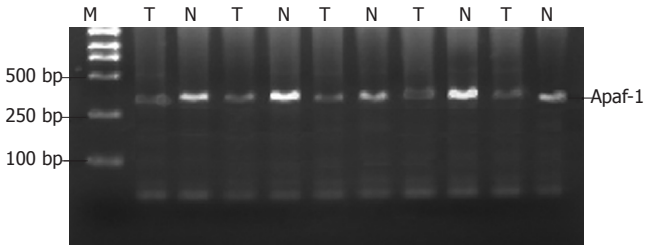


Figure 1 Expression of Apaf-1 mRNA. M: Marker, T: Tumor, N: Adjacent normal tissue, Apaf-1 mRNA: 334 bp, marker: DL2000.

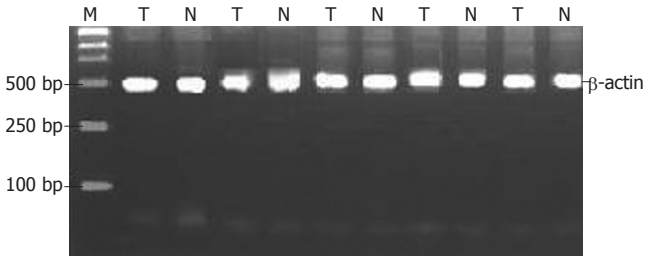


Figure 2 Expression of β-actin mRNA. M: Marker; T: Tumor; N: Normal adjacent tissue; β-actin mRNA: 498 bp; marker: DL2000.

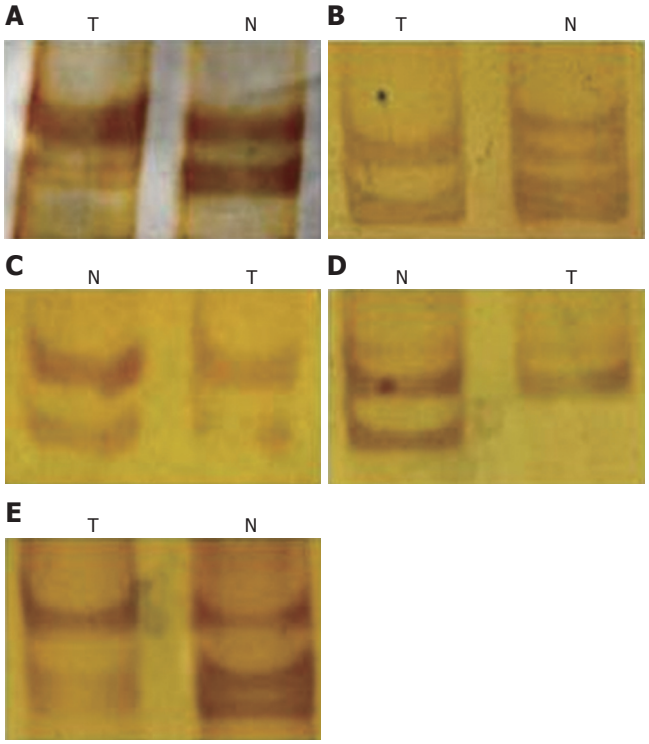


Figure 3 LOH analysis of polymorphic sites of D12S327 (**A**), D12S1657 (**B**), D12S393 (**C**), D12S1706 (**D**), and D12S346 (**E**). T: Tumor tissue; N: Adjacent normal tissue. Silver staining diagram showing Apaf-1 loss and LOH analysis showing allele loss at 5 polymorphic sites of Apaf-1 gene in gastric cancer tissue.

band with significantly weaker signal of up to 50% in cancer tissue samples than that in adjacent normal tissue samples. As shown by LOH analysis of 5 polymorphic sites for Apaf-1 gene in the present study, the LOH detection rate was 34.29% (12/35), 8.57% (3/35), 57.14% (20/35), 11.43% (4/35) and 42.86% (15/35) at single D12S346, D12S1706, D12S327, D12S1657 and

Table 2 Correlation of Apaf-1, LOH and Apaf-1 mRNA expression in gastric cancer tissue samples

		mRNA expression of Apaf-1 gene (*n*)		*r*s	*P*
		Down-regulation	Up-regulation		
More than 2 sites (*n*)	+	13	5	0.487	0.003
	-	4	13		

Table 3 Correlation of Apaf-1 methylation and Apaf-1 mRNA expression in gastric cancer tissue samples

		mRNA expression of Apaf-1 (*n*)		*r*s	*P*
		Down-regulation	Up-regulation		
Apaf-1 gene methylation (*n*)	+	16	1	0.886	10^{-6}
	-	1	17		

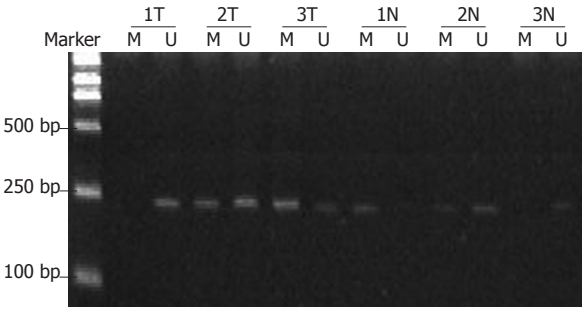


Figure 4 Methylation of Apaf-1 promoter. M: 212 bp methylation band; U: 221 bp non-methylation band; T: Gastric cancer tissue.

D12S393, respectively. However, the LOH detection rate was 51.43% (18/35) at more than 2 sites (16.67% at 3 sites) (Figure 3), suggesting that the LOH of Apaf-1 gene was correlated with the genesis of gastric cancer. Apaf-1 mRNA expression decreased significantly in 13 cases ($r_s = 0.487$, $P = 0.003$), indicating that the LOH of Apaf-1 gene was correlated with its decreased expression in gastric cancer (Table 2 and Figure 3).

Promoter methylation

MSP analysis of methylation conditions for Apaf-1 gene promoter showed that the methylation rate of Apaf-1 gene was 48.57% (17/35) in gastric cancer tissue samples and 17.14% (6/35) in adjacent normal tissue samples, respectively ($P < 0.05$), demonstrating that Apaf-1 methylation was correlated with the genesis of gastric cancer. Significant promoter methylation occurred in 16 of 18 gastric cancer tissue samples while the expression of Apaf-1 mRNA was decreased ($r_s = 0.886$, $P = 10^{-6}$), displaying that promoter methylation was correlated with decreased expression of Apaf-1 gene (Table 3 and Figure 4). Promoter methylation and LOH of Apaf-1 gene occurred at polymorphic sites in 17 of 18 gastric cancer tissue samples and the expression of Apaf-1 mRNA was decreased.

DISCUSSION

Human Apaf-1 gene is located at chromosome 12q23 to

encode cytoplasm protein (130 KD). As an important apoptosis factor, Apaf-1 gene participates in the pathway of mitochondria-mediated apoptosis[12]. However, the condition of Apaf-1 gene expression in gastric cancer and the correlation between expression of Apaf-1 gene and genesis of gastric cancer remain unclear. Although Apaf-1 is an important apoptosis factor and a tumor suppressor gene[12-16], few studies on the relationship between Apaf-1 gene and gastric cancer are available.

In the present study, Apaf-1 gene expression in gastric cancer tissue samples was significantly lower than that in adjacent normal tissue samples, suggesting that Apaf-1 gene plays an important role in genesis and progression of gastric cancer.

According to the epigenetics theory on gene regulation, methylation of gene fragments (especially promoter domain) inhibits gene transcription, and acetylation of gene-related histone up-regulates gene expression. One of the carcinogenic mechanisms is gene silencing caused by hyper-methylation of CpG islet at tumor suppressor gene promoter domain and deacetylation of histone[17-23]. It was reported that Apaf-1 gene is a tumor suppressor gene and seldom mutates, but has functional loss due to LOH and promoter methylation[24]. Apaf-1 gene has different effects and expression patterns in cancer tissue of different sources. The lower (or inactive) expression of Apaf-1 gene is related to methylation silencing in acute leukemia and laryngeal squamous carcinoma[24,25], and LOH in colon cancer, ovarian cancer and malignant melanocarcinoma[26-28].

In the present study, Apaf-1 gene promoter was methylated in 14 gastric cancer tissue samples with a decreased expression of Apaf-1 as detected by the MSP technique. Apaf-1 expression decreased in 13 gastric cancer tissue samples with LOH at more than 2 sites (including promoter methylation in 6 cases) as demonstrated by 9% non-denaturing PAGE. These findings show that decreased expression of Apaf-1 gene in gastric cancer tissue is due to some complex reasons, among which, however, promoter methylation and LOH at polymorphic sites play a major role.

COMMENTS

Background

The expression condition of Apaf-1 gene in gastric cancer and its correlation with the genesis of gastric cancer remain unclear. The purpose of this study was to make it clear.

Research frontiers

The peigenetics theory on gene regulation is the highlight in recent tumor studies. This article describes the role of Apaf-1 gene eigenetics in gastric cancer.

Innovations and breakthroughs

This article focuses on the Apaf-1 expression condition in gastric cancer, and its relation with loss of heterozygosity and methylation in promoter domain.

Applications

Apaf-1 gene is an important apoptosis gene. Its status of methylation and LOH may contribute to the study on the etiology of gastric cancer.

Terminology

Methylation is a term used in chemical sciences to denote the attachment or substitution of a methyl group on various substrates. DNA methylation profiling is gaining momentum as an epigenetic approach to understanding the effects of aberrant methylation (either hyper- or hypomethylation) both in basic research and in clinical applications.

Peer review

Low expression of Apaf-1 gene in gastric cancer tissue was shown in this study, indicating that methylation of Apaf-1 gene promoter and LOH in domain of 12q22-23 are the main reasons for expression and altered expression of Apaf-1 gene in gastric cancer

REFERENCES

1 **Mitsunaga M**, Tsubota A, Nariai K, Namiki Y, Sumi M, Yoshikawa T, Fujise K. Early apoptosis and cell death induced by ATX-S10Na (II)-mediated photodynamic therapy are Bax- and p53-dependent in human colon cancer cells. *World J Gastroenterol* 2007; **13**: 692-698

2 **Wang X**, Ye Z, Zhong J, Xiang J, Yang J. Adenovirus-mediated Il-24 expression suppresses hepatocellular carcinoma growth via induction of cell apoptosis and cycling arrest and reduction of angiogenesis. *Cancer Biother Radiopharm* 2007; **22**: 56-63

3 **Chiang CT**, Way TD, Lin JK. Sensitizing HER2-overexpressing cancer cells to luteolin-induced apoptosis through suppressing p21(WAF1/CIP1) expression with rapamycin. *Mol Cancer Ther* 2007; **6**: 2127-2138

4 **Leo C**, Richter C, Horn LC, Schütz A, Pilch H, Höckel M. Expression of Apaf-1 in cervical cancer correlates with lymph node metastasis but not with intratumoral hypoxia. *Gynecol Oncol* 2005; **97**: 602-606

5 **Mustika R**, Budiyanto A, Nishigori C, Ichihashi M, Ueda M. Decreased expression of Apaf-1 with progression of melanoma. *Pigment Cell Res* 2005; **18**: 59-62

6 **Ekert PG**, Read SH, Silke J, Marsden VS, Kaufmann H, Hawkins CJ, Gerl R, Kumar S, Vaux DL. Apaf-1 and caspase-9 accelerate apoptosis, but do not determine whether factor-deprived or drug-treated cells die. *J Cell Biol* 2004; **165**: 835-842

7 **Anichini A**, Mortarini R, Sensi M, Zanon M. APAF-1 signaling in human melanoma. *Cancer Lett* 2006; **238**: 168-179

8 **Ho CK**, Bush JA, Li G. Tissue-specific regulation of Apaf-1 expression by p53. *Oncol Rep* 2003; **10**: 1139-1143

9 **Sturm I**, Bosanquet AG, Radetzki S, Hummel M, Dörken B, Daniel PT. Silencing of APAF-1 in B-CLL results in poor prognosis in the case of concomitant p53 mutation. *Int J Cancer* 2006; **118**: 2329-2336

10 **Marsden VS**, O'Connor L, O'Reilly LA, Silke J, Metcalf D, Ekert PG, Huang DC, Cecconi F, Kuida K, Tomaselli KJ, Roy S, Nicholson DW, Vaux DL, Bouillet P, Adams JM, Strasser A. Apoptosis initiated by Bcl-2-regulated caspase activation independently of the cytochrome c/Apaf-1/caspase-9 apoptosome. *Nature* 2002; **419**: 634-637

11 **Sobin LH**, Fleming ID. TNM Classification of Malignant Tumors, fifth edition (1997). Union Internationale Contre le Cancer and the American Joint Committee on Cancer. *Cancer* 1997; **80**: 1803-1804

12 **Zou H**, Henzel WJ, Liu X, Lutschg A, Wang X. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. *Cell* 1997; **90**: 405-413

13 **Zlobec I**, Minoo P, Baker K, Haegert D, Khetani K, Tornillo L, Terracciano L, Jass JR, Lugli A. Loss of APAF-1 expression is associated with tumour progression and adverse prognosis in colorectal cancer. *Eur J Cancer* 2007; **43**: 1101-1107

14 **Deng WG**, Kawashima H, Wu G, Jayachandran G, Xu K, Minna JD, Roth JA, Ji L. Synergistic tumor suppression by coexpression of FUS1 and p53 is associated with down-regulation of murine double minute-2 and activation of the apoptotic protease-activating factor 1-dependent apoptotic pathway in human non-small cell lung cancer cells. *Cancer Res* 2007; **67**: 709-717

15 **Christoph F**, Kempkensteffen C, Weikert S, Köllermann J, Krause H, Miller K, Schostak M, Schrader M. Methylation of tumour suppressor genes APAF-1 and DAPK-1 and in vitro effects of demethylating agents in bladder and kidney cancer. *Br J Cancer* 2006; **95**: 1701-1707

16 **Zlobec I**, Steele R, Michel RP, Compton CC, Lugli A, Jass JR. Scoring of p53, VEGF, Bcl-2 and APAF-1 immunohistochemistry and interobserver reliability in colorectal cancer. *Mod Pathol* 2006; **19**: 1236-1242
17 **Roman-Gomez J**, Jimenez-Velasco A, Barrios M, Prosper F, Heiniger A, Torres A, Agirre X. Poor prognosis in acute lymphoblastic leukemia may relate to promoter hypermethylation of cancer-related genes. *Leuk Lymphoma* 2007; **48**: 1269-1282
18 **Ye M**, Xia B, Guo Q, Zhou F, Zhang X. Association of diminished expression of RASSF1A with promoter methylation in primary gastric cancer from patients of central China. *BMC Cancer* 2007; **7**: 120
19 **Karpf AR**. Epigenomic reactivation screening to identify genes silenced by DNA hypermethylation in human cancer. *Curr Opin Mol Ther* 2007; **9**: 231-241
20 **Lindsey JC**, Lusher ME, Anderton JA, Gilbertson RJ, Ellison DW, Clifford SC. Epigenetic deregulation of multiple S100 gene family members by differential hypomethylation and hypermethylation events in medulloblastoma. *Br J Cancer* 2007; **97**: 267-274
21 **Ren Y**, Liu X, Ma D, Feng Y, Zhong N. Down-regulation of the progesterone receptor by the methylation of progesterone receptor gene in endometrial cancer cells. *Cancer Genet Cytogenet* 2007; **175**: 107-116
22 **Watanabe T**, Katayama Y, Yoshino A, Yachi K, Ohta T, Ogino A, Komine C, Fukushima T. Aberrant hypermethylation of p14ARF and O6-methylguanine-DNA methyltransferase genes in astrocytoma progression. *Brain Pathol* 2007; **17**: 5-10
23 **Nelson WG**, Yegnasubramanian S, Agoston AT, Bastian PJ, Lee BH, Nakayama M, De Marzo AM. Abnormal DNA methylation, epigenetics, and prostate cancer. *Front Biosci* 2007; **12**: 4254-4266
24 **Huang DF**, Fu WN, Shang C, Xu ZM, Li ZG, Sun KL. Expression and promoter methylation of Apaf-1 gene in laryngeal squamous cell carcinoma. *Yichuan Xuebao* 2004; **31**: 1327-1331
25 **Fu WN**, Bertoni F, Kelsey SM, McElwaine SM, Cotter FE, Newland AC, Jia L. Role of DNA methylation in the suppression of Apaf-1 protein in human leukaemia. *Oncogene* 2003; **22**: 451-455
26 **Murty VV**, Montgomery K, Dutta S, Bala S, Renault B, Bosl GJ, Kucherlapati R, Chaganti RS. A 3-Mb high-resolution BAC/PAC contig of 12q22 encompassing the 830-kb consensus minimal deletion in male germ cell tumors. *Genome Res* 1999; **9**: 662-671
27 **Umetani N**, Fujimoto A, Takeuchi H, Shinozaki M, Bilchik AJ, Hoon DS. Allelic imbalance of APAF-1 locus at 12q23 is related to progression of colorectal carcinoma. *Oncogene* 2004; **23**: 8292-8300
28 **Baldi A**, Santini D, Russo P, Catricalà C, Amantea A, Picardo M, Tatangelo F, Botti G, Dragonetti E, Murace R, Tonini G, Natali PG, Baldi F, Paggi MG. Analysis of APAF-1 expression in human cutaneous melanoma progression. *Exp Dermatol* 2004; **13**: 93-97

S- Editor Zhu LH **L- Editor** Wang XL **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5065-5070
World Journal of Gastroenterology ISSN 1007-9327

BASIC RESEARCH

Modifications produced by selective inhibitors of cyclooxygenase and ultra low dose aspirin on platelet activity in portal hypertension

Francisco X Eizayaga, Omar Aguejouf, Vanessa Desplat, Philippe Belon, Christian Doutremepuich

Francisco X Eizayaga, CEBBAD, Universidad Maimónides, Buenos Aires, Argentina, France
Omar Aguejouf, Vanessa Desplat, Christian Doutremepuich, Laboratoire d'Hématologie, Université Victor Segalen, Bordeaux 2, Bordeaux, 146, Rue Léo Saignat, 33076 Bordeaux Cedex, France
Philippe Belon, Laboratoires Boiron, Sainte-Foy-les-Lyons, France
Correspondence to: Christian Doutremepuich, Laboratoire d' Hématologie, Université Victor Segalen, Bordeaux 2, 146, Rue Léo Saignat, 33076 Bordeaux Cedex,
France. christian.doutremepuich@heph.u-bordeaux2.fr
Telephone: +33-5-56987969 Fax: +33-5-56987970
Received: June 6, 2007 Revised: June 21, 2007

Abstract

AIM: To study the mechanism involved in the potentially beneficial effect of ultra low dose aspirin (ULDA) in prehepatic portal hypertension, rats were pretreated with selective COX 1 or 2 inhibitors (SC-560 or NS-398 respectively), and subsequently injected with ULDA or placebo.

METHODS: Portal hypertension was induced by portal vein ligation. Platelet activity was investigated with an *in-vivo* model of laser induced thrombus production in mesenteric circulation and induced hemorrhagic time (IHT). Platelet aggregation induced by ADP and dosing of prostanoid products 6-keto-$PGF_{1\alpha}$, TXB_2, PGE_2 and LTB_4 were also performed.

RESULTS: The portal hypertensive group receiving a placebo showed a decreased *in vivo* platelet activity with prolonged IHT, an effect that was normalized by ULDA. SC-560 induced a mild antithrombotic effect in the normal rats, and an unmodified effect of ULDA. NS-398 had a mild prothrombotic action in portal hypertensive rats, similar to ULDA, but inhibited a further effect when ULDA was added. An increased 6-keto-$PGF_{1\alpha}$ was observed in portal hypertensive group that was normalised after ULDA administration. TXA_2 level after ULDA, remained unchanged.

CONCLUSION: These results suggest that the effect of ULDA on platelet activity in portal hypertensive rats, could act through a COX 2 pathway more than the COX 1, predominant for aspirin at higher doses.

Key words: Ultra Low Dose Aspirin; Portal hypertension; COX 1; COX 2; COX selective inhibition

Eizayaga FX, Aguejouf O, Desplat V, Belon P, Doutremepuich C. Modifications produced by selective inhibitors of cyclooxygenase and ultra low dose aspirin on platelet activity in portal hypertension. *World J Gastroenterol* 2007; 13(38): 5065-5070

http://www.wjgnet.com/1007-9327/13/5065.asp

INTRODUCTION

Portal hypertension is a major complication of chronic liver disease. In its pathophysiology, increased hepatic resistance is followed by a hyperdynamic circulatory state[1]. This hyperdynamic state, in which nitric oxide (NO) and prosatcyclin (PGI_2) are important vasoactive substances, induces a decreased platelet activity, even in the absence of hepatic damage. Although NO plays an important role in modifying platelet adhesion in portal hypertension[2], PGI_2, a powerful vasodilator prostanoid with antithrombotic properties, was also found increased in mesenteric vascular bed[3].

Ultra low dose aspirin (ULDA) has shown prothrombotic activity when analysed in humans and in the interface platelet-endothelial cell[4,5]. Previous papers have shown the potentially beneficial effect of ULDA in portal hypertensive animals normalizing altered platelet activity and induced hemorrhagic time[6]. Further experiments were performed to clarify if this effect was mediated mostly by a cyclooxigenase (COX) pathway or by modifying NO synthesis, the two aspirin mechanisms of action[7], although a previous study with a different model suggested that ULDA could decrease PGI_2 synthesis[8]. The same *in vivo* Laser induced thrombus formation, was used in portal hypertensive rats to investigate the effects of Indomethacin, a non selective COX inhibitor, and L-Nitro Arginin Methyl Ester (NAME), a non selective inhibitor of NO production. The results suggested that the effects of ULDA were more influenced by COX pathway than by NO synthesis inhibition[9]. Addition of ULDA in portal hypertensive rats, when previously inhibiting COX with Indomethacin, increased number of emboli and duration

of embolization but blunted the normalization of induced hemorrhagic time, suggesting probably a more selective action on COX 1 or COX 2 pathway. TXA_2, the main product of arachidonic acid *via* the activity of COX 1 in platelets, increases platelet aggregation and its synthesis is inhibited by Aspirin. PGE_2 and PGI_2 are produced by COX 1 and 2 mainly in endothelial cells. PGE_2 has no probable role in portal hemodynamic changes observed in portal hypertensive rats[10], but depending on its concentration, it can modulate platelet aggregation by regulating intracellular levels of cAMP[11].

The present investigation was designed to clarify the mechanism of the effects of ULDA in portal hypertension, by using previous selective inhibition of cyclooxygenase COX 1 or COX 2 (with SC-560 and NS-398 respectively). Models of Laser induced Thrombosis and IHT were used and plasmatic levels of 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2), PGE_2, TXB_2 (the stable metabolite of TXA_2) and LTB_4 were determined.

MATERIALS AND METHODS

Animals

Male Wistar rats (200-250g) purchased from Delpre Breeding Center (St. Doulchard, France) were housed separately and acclimatized before use under conditions of controlled temperature (25 ± 2℃) and illumination (12 h light/dark cycle). They were fed with standard rat chow and water *ad libitum*. Animals received care in compliance with the European Convention of Animal Care.

Surgical procedures

After one week of acclimatization, rats were randomized and separated into two groups: one consisted of sham-operated (Sh) rats and the other formed by portal hypertensive (PH) rats. Portal hypertension was induced by a calibrated portal vein stenosis, according to the procedure described by Vorobioff *et al*[1].

Rats were anesthetized with Ketamine (Panpharma, Fougères, France) 90 mg/kg body weight, i.m. and then a midline abdominal incision was made. The portal vein was located and isolated from the surrounding tissues. A ligature of 3-0 silk was placed around the vein and snugly tied to a 20 gauge blunt end needle placed along side the portal vein. The needle was subsequently removed to yield a calibrated stenosis of the portal vein. Sham-operated rats underwent an identical procedure except that portal vein was isolated but not stenosed.

Animals were housed during fourteen days after the operation to develop portal hypertension in the corresponding group.

Thrombus induction

Animals were anaesthetized with 200 mg/kg of thiopental sodium (Pentothal®, Laboratories Abbott, Rungis, France) a median laparotomy was performed. The intestinal loop was placed on the microscope table and vascular lesions were induced by Argon laser (Stabilite 2016, Spectra Physics, France). The wavelength used was 514 nm and the energy was adjusted to 120 mW. The laser beam was applied during 1/15 s. The dynamic-course of thrombus formation was continuously monitored and recorded by placing the laser beam coaxially into the inverted light beam path of the microscope (Axiovert, Zeiss, France). Microscopic images were recorded through a digital camera (DX L107, color camera CCD, Basler, Vision Technologies) to visualize and digitalize data coupled to a Dell monitor. A schematic view of the apparatus used has been previously described[12]. Arterioles between 15 and 25 μm diameter were used. The parameters assessed were the number of emboli removed by blood flow and the duration of embolisation (time between the first and the last emboli occurring during a 10 min observation period).

Induced hemorrhagic time

An experimental model of induced hemorrhagic time (IHT) was performed 10 min before thrombosis induction by laser. The tail of the rat was immersed in water for 5 min at 37℃ and sectioned 6 mm from the extremity. IHT measured, corresponded to the time between the tail section and the end of bleeding, expressed in seconds.

Biological analysis

Platelet aggregation study: Platelet aggregation was made according to the method of Cardinal and Flower on a Chrono Log 500 *VS* aggregometer (Coultronics, Margency, France) on the whole blood obtained from the rat after laser experimentation. Platelet aggregation was induced by ADP final concentration 5 μmol/L (Laboratoire Diagnostica, Stago, France). Two parameters were determined: Impedance, representing the maximum amplitude of aggregation expressed in Ohms. Velocity of aggregation expressed in ohms/min.

Prostanoids determinations: At the end of each experiment, blood was collected by cardiac puncture and centrifuged for 20 min at 4000 r/min to obtain Platelet Poor Plasma (PPP). Concentrations of 6-keto-$PGF_{1\alpha}$, TXB_2, PGE_2 and LTB_4 were determined in plasma samples using competitive binding Elisa tests (R&D Systems Europe, Abingdon, UK) according to the manufacturer's instructions.

Drugs tested

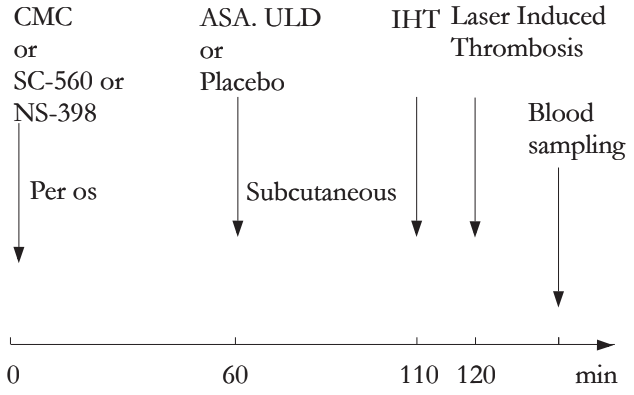
The Aspirin solution was purchased from Boiron Laboratories (Sainte-Foy-Les-Lyon, France). ULDA was prepared as follows: 1 g of pure, finely powdered aspirin was suspended in 99 mL of alcohol (70º). After being vigorously shaken, 1 mL of this dilution was then mixed with 99 mL of distilled water and vigorously shaken. The last process was repeated 13 more times[11]. Alcohol and sterilized water following the above cited procedures without adding the Aspirin was used as control. ULDA or placebo were subcutaneously administered at a final volume of 1 mL/kg rat weight.

Selective inhibitors of COX 1, SC-560 and of COX 2, NS-398 were purchased from Cayman Chemical, (Ann Arbor Michigan, USA). They were administered per os at a dose of 10 mg/kg rat weight, suspended in Carboxymethylcellulose (CMC) 5 g/L at a final volume

of 1 mL/kg rat weight. The CMC solution was used as placebo.

Protocol

Fourteen days after the corresponding operation, 216 rats were randomly assigned in 12 groups and treated as follows:



Groups

Groups, procedures and treatments are detailed in Table 1.

Statistical analysis

Data are expressed as mean ± SEM and compared using one way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test. A value of $P < 0.05$ was considered significant. Statistical calculations were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

RESULTS

Thrombus induction and IHT

Groups with placebo (CMC): Portal hypertension decreased the number of emboli ($P < 0.05$) and the duration of embolisation ($P < 0.05$) and prolonged the Induced Hemorrhagic Time ($P < 0.01$). ULDA induced significant modifications to normalize these values ($P < 0.01$, $P < 0.001$ and $P < 0.001$ for Number of Emboli, Duration of Embolisation and IHT respectively). An increased number of shots needed to start the embolisation was observed in the portal hypertensive group, and this effect also normalised after ULDA ($P < 0.05$).

Effects of SC-560, selective inhibitor of COX 1: After the inhibition of COX 1, a non significant antithrombotic effect was observed in the sham operated group, and no further change in cirrhotic group. After ULDA, the prothrombotic effect remained active for Number of Emboli ($P < 0.05$) and IHT ($P < 0.01$) despite the opposite effect of pre-treatment with SC-560. No changes were observed in number of shots.

Effects of NS-398, selective inhibitor of COX 2: The pre-treatment with NS-398 induced a decreased induced hemorrhagic time in portal hypertensive rats, similar to the effect of ULDA without COX inhibitors. No effect of ULDA was seen after COX 2 inhibition (Figure 1A-D).

Table 1 Experimental groups

Group	Procedure and treatments		
	Phase 1: 14 d before treatments	Phase 2: 120 min before experiment	Phase 3: 60 min before experiment
ShP	Sham operated	Placebo (CMC)	Placebo (H_2O)
PHP	Portal hypertension	Placebo (CMC)	Placebo (H_2O)
PHPAs	Portal hypertension	Placebo (CMC)	ULDA
ShP	Sham operated	SC-560	Placebo (H_2O)
PHP	Portal hypertension	SC-560	Placebo (H_2O)
PHPAs	Portal hypertension	SC-560	ULDA
ShP	Sham operated	NS-398	Placebo (H_2O)
PHP	Portal hypertension	NS-398	Placebo (H_2O)
PHAs	Portal hypertension	NS-398	ULDA

ULDA: Ultra low dose aspirin; CMC: Carboxymethylcellulose; ShP: Sham-placebo; PHP: Portal hypertension-placebo; PHPAs: Portal hypertension-ULDA.

Biological analysis

Platelet aggregation induced by ADP (Figure 2A and B): Platelet aggregation has shown a decreased velocity in portal hypertensive animals and in sham operated animals pretreated with SC-560. An increased velocity was observed in portal hypertensive animals pretreated with SC-560 or NS-398 and with ULDA. None of the observed alterations were found in Amplitude and in Velocity.

Variations in metabolites of arachidonic acid (Figure 3A-D): 6 keto $PGF_{1\alpha}$ (Figure 3A): This stable metabolite of PGI_2 was found increased in portal hypertensive rats ($P < 0.01$). This increase was normalised by ULDA ($P < 0.05$) or SC-560 ($P < 0.05$).

TXB_2 (Figure 3B): Inhibition of COX 2 with NS-398 produced an increased level of TXB_2 in portal hypertensive animals (XI group), that was not modified by ULDA ($P < 0.05$ and 0.001 respectively).

PGE_2 (Figure 3C): An increase in PGE_2 was observed in portal hypertensive (PHP) group, treated with SC-560 ($P < 0.05$).

LTB_4 (Figure 3D): Dosage of LTB_4, a lipoxygenase (LOX) metabolite of arachidonic acid, was performed as control of experiment. No variations of production were found when comparing the different groups.

DISCUSSION

ULDA has shown prothrombotic effects with an *in vivo* model, testing the interaction between platelet and endothelial cells[5,12]. Exploration of this effect in portal hypertensive rats revealed a decreased number of emboli as well as duration of embolisation and a prolonged IHT that were normalised by ULDA administration. In the search of an explanation for the mechanism involving this effect, previous publications of L-NAME (an inhibitor of Nitric Oxide synthesis) and Indomethacin (a nonselective COX inhibitor) effects on portal hypertensive rats showed that the effect of ULDA was more affected by Indomethacin than by NAME[9]. Moreover, Indomethacin seemed to

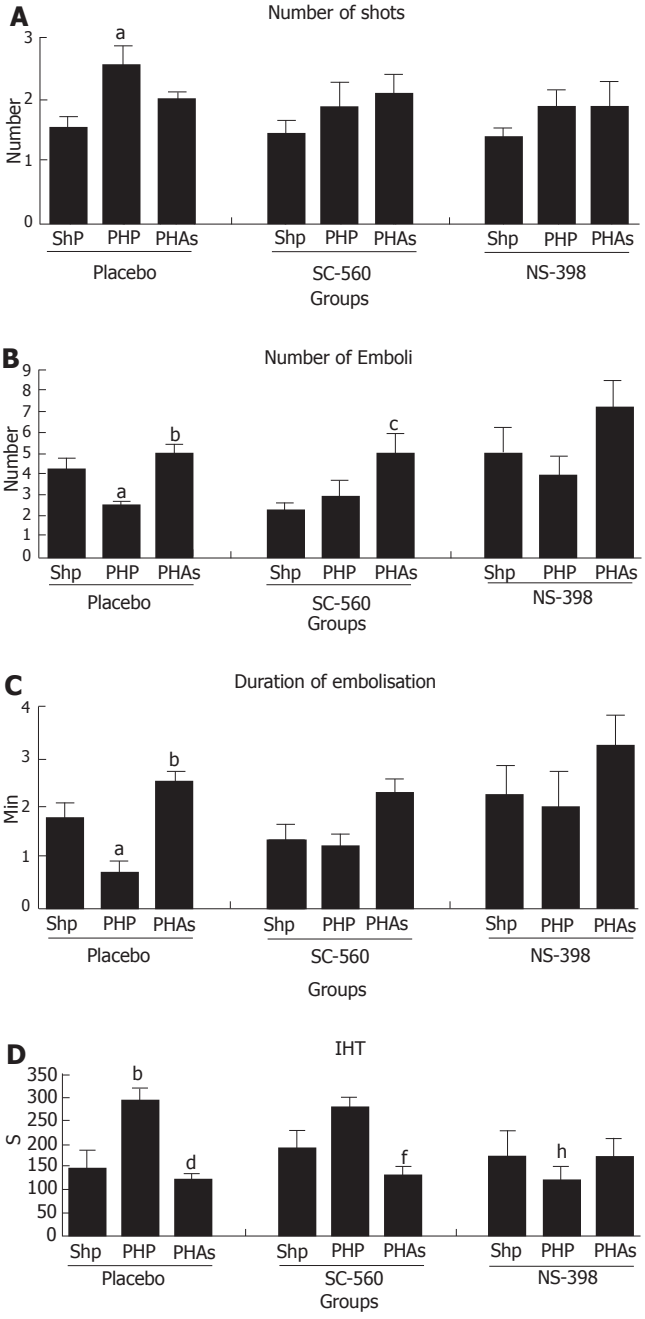


Figure 1 **A**: Study of laser induced thrombosis parameters; Number of shots (expressed in number): $^{a}P < 0.05$ *vs* ShP (Placebo); **B**: Study of laser induced thrombosis parameters; Number of Emboli (expressed in number): $^{a}P < 0.05$ *vs* ShP (placebo); $^{b}P < 0.01$ *vs* PHP (placebo); $^{c}P < 0.05$ *vs* ShP (SC-560); **C**: Study of laser induced thrombosis parameters; Duration of Embolisation (expressed in minutes): $^{a}P < 0.05$ *vs* ShP (placebo); $^{b}P < 0.001$ *vs* PHP (placebo); **D**: Study of Induced Hemorrhagic Time (expressed in seconds): $^{b}P < 0.01$ *vs* ShP (placebo); $^{d}P < 0.001$ *vs* PHP (placebo); $^{f}P < 0.01$ *vs* PHP (SC-560); $^{h}P < 0.001$ *vs* PHP (placebo).

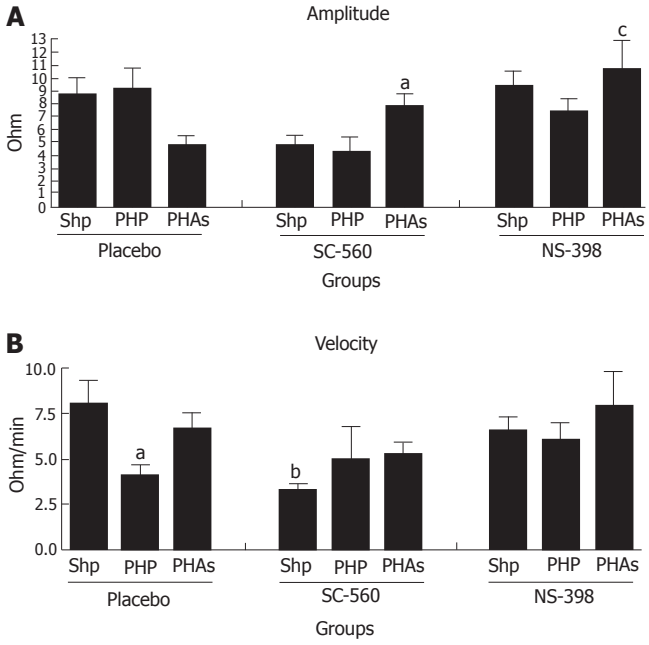


Figure 2 **A**: Study of Platelet aggregation induced by ADP, Amplitude (expressed in Ohm): $^{a}P < 0.05$ *vs* PHP (SC-560); $^{c}P < 0.05$ *vs* PHPAs (placebo); **B**: Study of Platelet aggregation induced by ADP, Velocity (expressed in Ohm/min): $^{a}P < 0.05$ *vs* ShP (placebo); $^{b}P < 0.01$ *vs* ShP (placebo).

have contradictory effects. Despite the antithrombotic effect of Indomethacin, the *in vivo* prothrombotic effect of ULDA (increasing number of emboli and duration of embolization) was increased, and its beneficial effect of reducing IHT in portal hypertensive rats was blocked. The present experiment was done to verify the hypothesis that these contradictory modifications produced by Indomethacin over ULDA's prothrombotic effect were the result of different ways in which COX 1 and 2 could affect platelet-endothelial cell interaction.

The results found in this study corroborate the previously described effects of ULDA in portal hypertensive rats, normalizing number of emboli, duration of embolisation and the IHT[6,9].

In rats with portal hypertension, an increase in 6-keto $PGF_{1\alpha}$ was observed, probably due to the known increased PGI_2 production described for the mesenteric vascular bed in this animal model[3]. The addition of ULDA normalised this effect. As reported in an *in vitro* model with a vascular fragment, ULDA was active only in vascular fragments with an elevated PGI_2 production[8]. This last observation is similar to our present results since the ULDA effect of decreasing 6-keto $PGF_{1\alpha}$ is observed only in the portal hypertensive group.

The administration of SC-560, had a slightly antithrombotic effect in sham operated rats, decreasing non significantly the Number of Emboli. This could be explained by a decrease in platelet synthesis of TXA_2[13]. There was a decrease in 6-keto $PGF_{1\alpha}$ in the portal hypertensive group with COX 1 inhibition, and this effect is probably due to the inhibition of the increased production of PGI_2[14,15] and COX 1 over-expression observed in this model of portal vein ligation[16-18]. It is interesting to note that COX 1 inhibition had almost not modified the prothrombotic effect of ULDA in portal hypertensive animals and observed as an increase in number of emboli ($P < 0.05$) and a decrease in IHT ($P < 0.01$).

The administration of NS-398 had not modified the *in vivo* parameters (NE, DE and IHT) in the Sham operated group. In the portal hypertensive group, a non significant tendency to increase NE and DE was observed, as well as a statistically significant shortened IHT. After pre-treatment

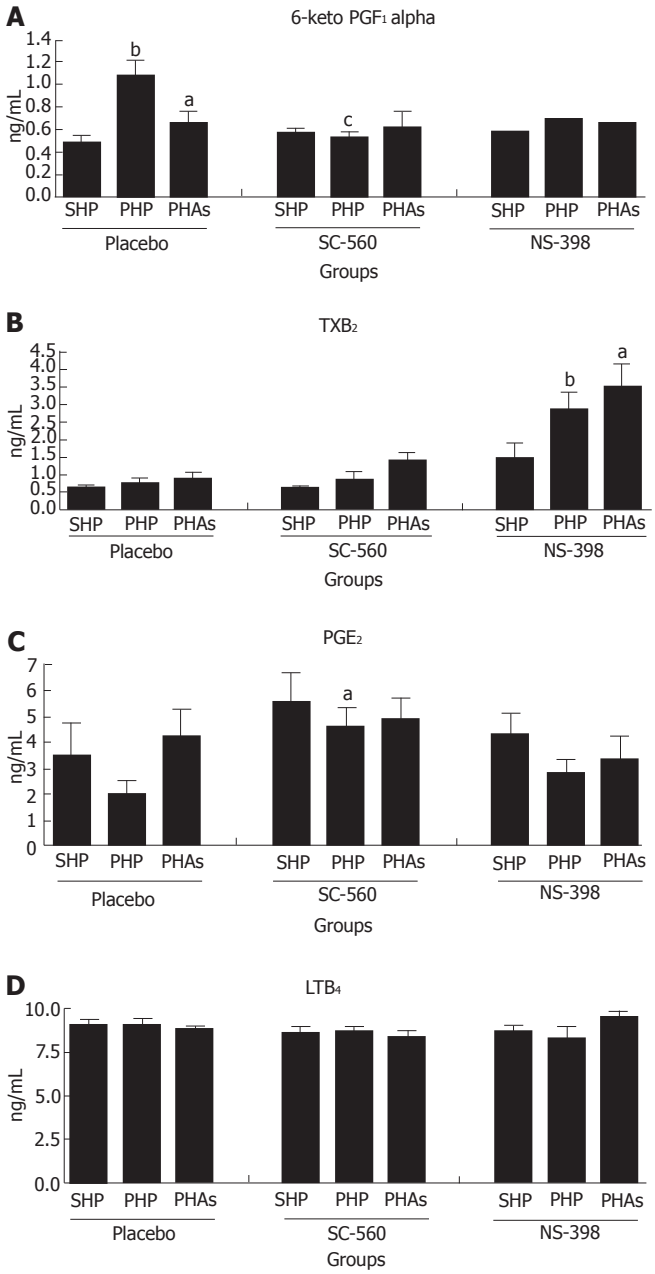


Figure 3 **A**: Plasmatic 6-keto $PGF_{1\alpha}$ concentrations (expressed in ng/mL): $^{b}P < 0.01$ *vs* ShP (placebo); $^{a}P < 0.05$ *vs* PHP (placebo); $^{c}P < 0.05$ *vs* PHP (placebo); **B**: Plasmatic TXB_2 concentrations (expressed in ng/mL): $^{b}P < 0.001$ *vs* PHP (placebo); $^{a}P < 0.05$ *vs* ShP (NS-398) and $P < 0.001$ *vs* PHAs (placebo); **C**: Plasmatic PGE_2 concentrations (expressed in ng/mL): $^{a}P < 0.05$ *vs* PHP (placebo); **D**: Plasmatic LTB_4 concentrations (expressed in ng/mL).

with the selective COX 2 inhibitor, NS-398, ULDA induced no further effect. There is an increase in TXB2 in the portal hypertensive group with COX 2 inhibition, in which a decreased IHT was observed. Factors like trauma-hemorrhage, shear stress and pressure variations or lipopolysaccharide can modify TXA_2 synthesis in the liver or in endothelial cells[19-21]. The increased TXB_2 was not modified by treatment with ULDA. It is interesting to have, in the portal hypertensive group, an increased PGE_2 after COX 1 inhibition and an increased TXB_2 after COX 2 inhibition, as if upon COX selective inhibition, the cell switched to a prostanoid produced by the non inhibited COX enzyme.

The effect of ULDA was confirmed in this study with a prehepatic portal hypertension with a normal liver. Further research will clarify if this potentially beneficial effect is produced in rats with cirrhosis and ascities. Other complex interactions can not be evaluated by this study. For example, a recent publication has pointed out that chronic COX inhibition with Indomethacin enhances the collateral vascular responsiveness to Arginin-Vasopressin, which is also able to activate platelets[22,23].

In conclusion, despite that COX 1 inhibition caused a mild antithrombotic effect in sham operated rats, the prothrombotic effect of ULDA was not modified. COX 2 inhibition induced a mild prothrombotic effect over portal hypertensive rats, similar to that observed with ULDA alone confirming data of literature[24] and the addition of ULDA in this group produced no further changes. ULDA induced a decrease in PGI_2 in portal hypertensive animals, without modifying TXA_2 levels. These results suggested a predominant COX 2 inhibition by ULDA, opposite to the predominant inhibition of COX 1 commonly observed with ASA in usual doses.

COMMENTS

Background

Ultra Low Dose Aspirin (ULDA) has shown prothrombotic properties capable of normalizing altered platelet function found in portal hypertension. This effect is clearly the opposite of the actual main use of Aspirin as an antithrombotic drug.

Research frontiers

The mechanism of this effect is unknown, but previous publications show changes in this effect after pretreatment with Indomethacin, a widely used non-selective COX inhibitor.

Innovation and breakthroughs

Only inhibition of NO synthesis, and perhaps Vasopressin has shown this property of modifying platelet alterations in portal hypertension.

Applications

ULDA could be useful in patients with prehepatic portal hypertension to control or decrease bleeding complications.

Peer review

Aspirin is widely known as a more powerful inhibitor of COX 1 than COX 2. This is the first paper showing a positive effect of Aspirin in portal hypertension, based in a not yet explained inhibition of COX 2, rather than COX 1.

REFERENCES

1 **Vorobioff J**, Bredfeldt JE, Groszmann RJ. Hyperdynamic circulation in portal-hypertensive rat model: a primary factor for maintenance of chronic portal hypertension. *Am J Physiol* 1983; **244**: G52-G57

2 **Albornoz L**, Bandi JC, Otaso JC, Laudanno O, Mastai R. Prolonged bleeding time in experimental cirrhosis: role of nitric oxide. *J Hepatol* 1999; **30**: 456-460

3 **Hamilton G**, Rosza I, Hutton R, Chow FP, Dandona P, Hobbs KE. Portal vein prostacyclin activity in experimental portal hypertension in rats. *Clin Sci* (Lond) 1981; **60**: 327-329

4 **Doutremepuich C**, de Sèze O, Le Roy D, Lalanne MC, Anne MC. Aspirin at very ultra low dosage in healthy volunteers: effects on bleeding time, platelet aggregation and coagulation. *Haemostasis* 1990; **20**: 99-105

5 **Lalanne MC**, Doutremepuich C, de Sèze O, Belon P. What is the effect of acetylsalicylic acid at ultra low dose on the

interaction platelets/vessel wall? *Thromb Res* 1990; **60**: 231-236
6 **Eizayaga FX**, Aguejouf O, Desplat V, Belon P, Doutremepuich C. Modifications produced by indomethacin and L-NAME in the effect of ultralow-dose aspirin on platelet activity in portal hypertension. *Pathophysiol Haemost Thromb* 2006; **35**: 357-363
7 **Taubert D**, Berkels R, Grosser N, Schröder H, Gründemann D, Schömig E. Aspirin induces nitric oxide release from vascular endothelium: a novel mechanism of action. *Br J Pharmacol* 2004; **143**: 159-165
8 **Lalanne MC**, Ramboer I, de Sèze O, Doutremepuich C. In vitro platelets/endothelial cells interactions in presence of acetylsalicylic acid at various dosages. *Thromb Res* 1992; **65**: 33-43
9 **Eizayaga FX**, Aguejouf O, Belon P, Doutremepuich C. Platelet aggregation in portal hypertension and its modification by ultra-low doses of aspirin. *Pathophysiol Haemost Thromb* 2005; **34**: 29-34
10 **Ackerman Z**, Karmeli F, Rachmilewitz D. Longitudinal prostaglandin E(2) generation in various organs during evolution of experimental portal hypertension. *Prostaglandins Leukot Essent Fatty Acids* 2002; **67**: 197-201
11 **Fabre JE**, Nguyen M, Athirakul K, Coggins K, McNeish JD, Austin S, Parise LK, FitzGerald GA, Coffman TM, Koller BH. Activation of the murine EP3 receptor for PGE2 inhibits cAMP production and promotes platelet aggregation. *J Clin Invest* 2001; **107**: 603-610
12 **Doutremepuich C**, Aguejouf O, Pintigny D, Sertillanges MN, De Seze O. Thrombogenic properties of ultra-low-dose of acetylsalicylic acid in a vessel model of laser-induced thrombus formation. *Thromb Res* 1994; **76**: 225-229
13 **Teng XW**, Davies NM. High-performance liquid chromatographic analysis of a selective cyclooxygenase-1 inhibitor SC-560 in rat serum: application to pharmacokinetic studies. *J Pharm Biomed Anal* 2004; **35**: 1143-1147
14 **Wu Y**, Burns C, Campbell KA, Sitzmann JV. Systemic and portal prostacyclin and thromboxane response to hemorrhage in portal hypertension. *Shock* 1994; **2**: 68-71
15 **Wu ZY**, Chen XS, Qiu JF, Cao H. Role of PGI2 in the formation and maintenance of hyperdynamic circulatory state of portal hypertensive rats. *World J Gastroenterol* 2005; **11**: 752-755
16 **Potenza MA**, Botrugno OA, De Salvia MA, Lerro G, Nacci C, Marasciulo FL, Andriantsitohaina R, Mitolo-Chieppa D. Endothelial COX-1 and -2 differentially affect reactivity of MVB in portal hypertensive rats. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G587-G594
17 **Huang HC**, Wang SS, Chen YC, Lee FY, Chang FY, Lin HC, Hou MC, Chan CC, Chen CT, Wu SL, Lee SD. Cyclooxygenase expression in splanchnic hyposensitivity to glypressin of bleeding portal hypertensive rats. *Eur J Clin Invest* 2003; **33**: 505-512
18 **Hou MC**, Cahill PA, Zhang S, Wang YN, Hendrickson RJ, Redmond EM, Sitzmann JV. Enhanced cyclooxygenase-1 expression within the superior mesenteric artery of portal hypertensive rats: role in the hyperdynamic circulation. *Hepatology* 1998; **27**: 20-27
19 **Bouaziz A**, de Ficquelmont-Loïzos MM, Richert A, Caprani A. Direct physical factors and PGI2 and TXA2 secretions by a human endothelial cell line: in vitro investigation of pressure and shear stress applied independently or in synergy. *Thromb Res* 1998; **90**: 279-289
20 **Yokoyama Y**, Toth B, Kitchens WC, Schwacha MG, Bland KI, Chaudry IH. Role of thromboxane in producing portal hypertension following trauma-hemorrhage. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G1293-G1299
21 **Bezugla Y**, Kolada A, Kamionka S, Bernard B, Scheibe R, Dieter P. COX-1 and COX-2 contribute differentially to the LPS-induced release of PGE2 and TxA2 in liver macrophages. *Prostaglandins Other Lipid Mediat* 2006; **79**: 93-100
22 **Wun T**, Paglieroni T, Lachant NA. Physiologic concentrations of arginine vasopressin activate human platelets in vitro. *Br J Haematol* 1996; **92**: 968-972
23 **Huang HC**, Wang SS, Chen YC, Lee FY, Chang FY, Lin HC, Hou MC, Chang CC, Lee SD. Chronic cyclooxygenase blockade enhances the vasopressin responsiveness in collaterals of portal hypertensive rats. *Scand J Gastroenterol* 2006; **41**: 1440-1445
24 **Buerkle MA**, Lehrer S, Sohn HY, Conzen P, Pohl U, Krötz F. Selective inhibition of cyclooxygenase-2 enhances platelet adhesion in hamster arterioles in vivo. *Circulation* 2004; **110**: 2053-2059

S- Editor Liu Y **L- Editor** Alpini GD **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5071-5078
World Journal of Gastroenterology ISSN 1007-9327

BASIC RESEARCH

Induction of ischemic tolerance in rat liver *via* reduced nicotinamide adenine dinucleotide phosphate oxidase in Kupffer cells

Kazuaki Tejima, Masahiro Arai, Hitoshi Ikeda, Tomoaki Tomiya, Mikio Yanase, Yukiko Inoue, Takako Nishikawa, Naoko Watanabe, Natsuko Ohtomo, Masao Omata, Kenji Fujiwara

Kazuaki Tejima, Hitoshi Ikeda, Tomoaki Tomiya, Mikio Yanase, Yukiko Inoue, Takako Nishikawa, Naoko Watanabe, Natsuko Ohtomo, Masao Omata, Department of Gastroenterology, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
Masahiro Arai, Department of Gastroenterology, Toshiba General Hospital, 6-3-22 Higashiooi, Shinagawa-ku, Tokyo 140-8522, Japan
Kenji Fujiwara, Yokohama Rosai Hospital, 3211 Kozukue-cho, Kouhoku-ku, Yokohama-shi, Kanagawa 222-0036, Japan
Supported in part by Grant-in-Aid for Scientific Research No. 17590615 to K. T. from Japan Society for the Promotion of Science
Correspondence to: Masahiro Arai, MD, PhD, Department of Gastroenterology, Toshiba General Hospital, 6-3-22 Higashiooi, Shinagawa-ku, Tokyo 140-8522, Japan. araima-tky@umin.ac.jp
Telephone: +81-3-37640511 Fax: +81-3-37643415
Received: May 12, 2007 Revised: July 28, 2007

Abstract

AIM: To elucidate the mechanisms of hepatocyte preconditioning by H_2O_2 to better understand the pathophysiology of ischemic preconditioning.

METHODS: The *in vitro* effect of H_2O_2 pretreatment was investigated in rat isolated hepatocytes subjected to anoxia/reoxygenation. Cell viability was assessed with propidium iodide fluorometry. In other experiments, rat livers were excised and subjected to warm ischemia/reperfusion in an isolated perfused liver system to determine leakage of liver enzymes. Preconditioning was performed by H_2O_2 perfusion, or by stopping the perfusion for 10 min followed by 10 min of reperfusion. To inhibit Kupffer cell function or reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, gadolinium chloride was injected prior to liver excision, or diphenyleneiodonium, an inhibitor of NADPH oxidase, was added to the perfusate, respectively. Histological detection of oxygen radical formation in Kupffer cells was performed by perfusion with nitro blue tetrazolium.

RESULTS: Anoxia/reoxygenation decreased hepatocyte viability compared to the controls. Pretreatment with H_2O_2 did not improve such hepatocyte injury. In liver perfusion experiments, however, H_2O_2 preconditioning reduced warm ischemia/reperfusion injury, which was reversed by inhibition of Kupffer cell function or NADPH oxidase. Histological examination revealed that H_2O_2 preconditioning induced oxygen radical formation in Kupffer cells. NADPH oxidase inhibition also reversed hepatoprotection by ischemic preconditioning.

CONCLUSION: H_2O_2 preconditioning protects hepatocytes against warm ischemia/reperfusion injury *via* NADPH oxidase in Kupffer cells, and not directly. NADPH oxidase also mediates hepatoprotection by ischemic preconditioning.

Key words: Diphenyleneiodonium chloride; Ischemia/reperfusion injury; Ischemic preconditioning; Liver transplantation; Oxygen radicals

Tejima K, Arai M, Ikeda H, Tomiya T, Yanase M, Inoue Y, Nishikawa T, Watanabe N, Ohtomo N, Omata M, Fujiwara K. Induction of ischemic tolerance in rat liver *via* reduced nicotinamide adenine dinucleotide phosphate oxidase in Kupffer cells. *World J Gastroenterol* 2007; 13(38): 5071-5078

http://www.wjgnet.com/1007-9327/13/5071.asp

INTRODUCTION

The interruption of hepatic blood flow followed by reperfusion, called ischemia/reperfusion, causes liver injury in a number of clinical interventions such as liver transplantation, hepatic resection, abdominal surgery with hepatic vascular occlusion, and hypovolemic shock[1-4]. Especially in liver transplantation, ischemia/reperfusion injury can lead to the primary dysfunction of liver allografts; still the most feared complication after liver transplantation because of the associated high mortality, and the fact that there is no treatment other than retransplantation[5-7]. The pathophysiology and substantial mechanisms of hepatic ischemia/reperfusion injury are increasingly well understood, and recent studies have drawn attention to ischemic preconditioning as one therapeutic strategy to decrease any injury[4,8-10]. Ischemic preconditioning is a phenomenon by which brief periods of ischemia followed by reperfusion render tissues

resistant to subsequent prolonged ischemia/reperfusion. This phenomenon was originally characterized in a canine model of myocardial ischemia[11], and has since been recognized in hepatic injury after ischemia/reperfusion in animal models[12-14], as well as in humans[15-18].

We have recently reported that hepatoprotection of ischemic preconditioning in rats is mediated by reactive oxygen species (ROS) produced by Kupffer cells. Moreover, we have shown that hepatic pretreatment with a sublethal dose of H_2O_2 mimics the hepatoprotective effect of ischemic preconditioning[19]. Since H_2O_2 is produced as a consequence of ROS metabolism, it may be possible that H_2O_2 resulting from ROS production in Kupffer cells directly affects hepatocytes, and induces their adaptive response to ischemia/reperfusion injury. Therefore, in this study we sought to elucidate the mechanisms of H_2O_2 preconditioning to better understand the pathophysiology of ischemic preconditioning. We report here that H_2O_2 preconditioning did not protect hepatocytes directly, but via Kupffer cells, as in ischemic preconditioning. We also noted that the activation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in Kupffer cells may play an important role in hepatoprotection of H_2O_2, as well as in ischemic preconditioning.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing 180-240 g were maintained on a commercial pellet diet and water *ad libitum*, in a room under normal lighting conditions. All animals received humane care in compliance with our institutional and National Institutes of Health guidelines.

In vitro experiments

Isolation of hepatocytes: Rat hepatocytes were isolated and cultured as previously described[20]. Rat livers were minced after perfusion with 0.8 mg/mL collagenase (type I; Worthington Biochemical Corporation, Lakewood, NJ, USA) through the portal vein. Hepatocytes were separated from non-parenchymal cells by centrifugation at 50 × *g* for 2 min at 4℃. The viability of the collected hepatocytes was ≥ 95%, as determined by the trypan blue exclusion test. Hepatocytes were resuspended in William's E medium containing 100 mL/L fetal calf serum, 27 mmol/L $NaHCO_3$, 100 nmol/L insulin, and 10 nmol/L dexamethasone at pH 7.4. Hepatocytes were then plated on type I collagen-coated 96-multiwell plates (Asahi Techno Glass Corporation, Tokyo, Japan) at a density of 4×10^4 cells/well and incubated overnight in an atmosphere of 95% air/5% CO_2 at 37℃.

Treatment of isolated hepatocytes: Isolated hepatocytes were treated by incubation for 10 min at 37℃ with 100 μL Krebs-Ringer N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer (KRH) at pH 7.4 containing various concentrations of H_2O_2, and washed twice with KRH. We set doses of H_2O_2 up to 1 mmol/L in accordance with earlier liver perfusion experiments[19]. Hepatocytes were then incubated in an anaerobic chamber under anoxic conditions using an AnaeroPack (Mitsubishi Gas Chemical Corporation, Tokyo, Japan), and subsequently subjected to reoxygenation at 37℃. The oxygen concentration in the chamber decreased to < 0.005% within 30 min after insertion of AnaeroPack. Control hepatocytes were cultured under normoxic conditions for the same length of time at 37℃.

Cell viability assay: Cell viability was determined by propidium iodide fluorometry as previously described, with some modification[21]. Hepatocytes were incubated in 100 μL KRH containing 30 μmol/L propidium iodide, after H_2O_2 treatment. Fluorescence from each well was measured immediately before anoxic incubation and then at given times after reoxygenation. Cell viability after reoxygenation was calculated, with final fluorescence corresponding to the 100% cell death obtained by addition of 350 μmol/L digitonin. We used a Cytofluor S4000 fluorescence reader (Applied Biosystems, Stafford, TX, USA), employing 530 nm excitation and 645 nm emission filters.

Liver perfusion experiments

Preconditioning before warm ischemia/reperfusion injury: Rats were anesthetized intraperitoneally with 50 mg/kg pentobarbital sodium, and the abdomen was opened. Livers were perfused through the portal vein with Krebs-Henseleit bicarbonate buffer (KHB) saturated with 95% O_2 and 5% CO_2 at 30 mL/min for 10 min at 37℃. For H_2O_2 preconditioning, livers were perfused with KHB containing 1 mmol/L H_2O_2 for 10 min, and then perfused with KHB for 2 min to wash out H_2O_2, as previously described[19]. For ischemic preconditioning, the perfusion was stopped for 10 min, followed by reperfusion for 10 min. In control rats, livers were manipulated similarly except for adding H_2O_2 or stopping the perfusion, respectively.

Drug treatments: In some rats, 20 mg/kg gadolinium chloride ($GdCl_3$; Wako Pure Chemical Industries, Tokyo, Japan) was injected intravenously at 24 h before H_2O_2 preconditioning, under light ether anesthesia to inhibit Kupffer cell function[22,23]. $GdCl_3$ was dissolved in saline, and control rats were injected with the appropriate vehicle solution. The efficacy of $GdCl_3$ injection was confirmed by immunohistochemical staining of liver sections with anti-ED2 antibody in the preliminary experiments. In other rats, diphenyleneiodonium chloride (DPI; Sigma-Aldrich, St. Louis, MO, USA), an inhibitor of NADPH oxidase, at a final concentration of 10 μmol/L, was added to the perfusate[24] during H_2O_2 preconditioning, or the reperfusion period after ischemic preconditioning. Livers were then perfused with KHB for 10 min to wash out DPI. DPI was dissolved in dimethyl sulfoxide (DMSO) at 20 mmol/L. Livers of vehicle-treated rats were perfused with KHB containing 500 μL/L DMSO during each preconditioning.

Warm ischemia/reperfusion: After H_2O_2 preconditioning or ischemic preconditioning, livers were stored in KHB

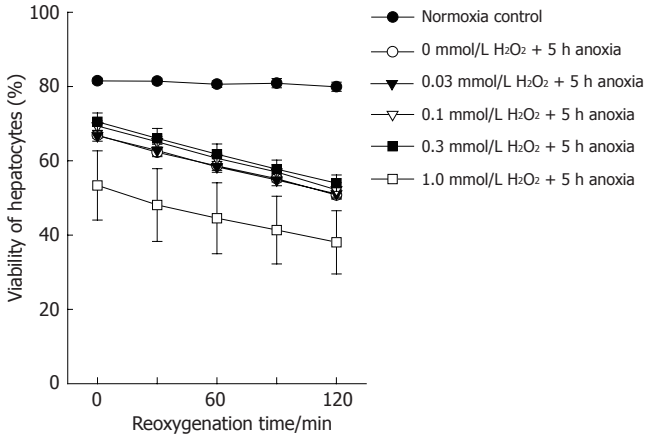


Figure 1 Viability of hepatocytes after anoxia/reoxygenation. (●): Viability of hepatocytes cultured under normoxia consistently. Viability of hepatocytes, pretreated with (○): 0 mmol/L; (▼): 0.03 mmol/L; (▽): 0.1 mmol/L; (■): 0.3 mmol/L; or (□): 1.0 mmol/L of H_2O_2 for 10 min before anoxia/reoxygenation.

at 37℃ as previously described[19]. After 40 min storage, livers were reperfused in a recirculating system with 200 mL KHB saturated with 95% O_2 and 5% CO_2 at 37℃. The perfusate was collected after 60 min of reperfusion for determination of alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) activity.

Biochemical assays: Commercial kits (Wako Pure Chemical Industries) were used to determine ALT and LDH activity in the perfusates.

Determination of oxygen radical formation in Kupffer cells after H_2O_2 preconditioning

Livers were perfused through the portal vein with KHB for 10 min, and then perfused with KHB containing 1 mmol/L H_2O_2 for 10 min. Subsequently, the livers were perfused with KHB containing 500 mg/L nitro blue tetrazolium (NBT; Sigma-Aldrich) for 10 min. Livers were then fixed by infusion of 10% formalin, embedded in paraffin, sectioned and stained with nuclear fast red. As formazan deposition is formed by the reaction of NBT with oxygen radicals in Kupffer cells, the number of formazan-positive cells was determined as the mean in 10 different areas of each section, observed by light microscopy at × 400 magnification (high power field).

Statistical analysis

All values are expressed as means ± SE. The difference between the means was analyzed with Student's *t* test, after confirming that the data passed normal distribution and equal-variance tests. $P < 0.05$ was considered significant.

RESULTS

Effect of H_2O_2 preconditioning on anoxia/reoxygenation injury of isolated hepatocytes

In the preliminary experiments, the viability of hepatocytes exposed to anoxia for > 3-4 h decreased after reoxygenation, compared to cells consistently cultured under normoxic conditions. Anoxic culture for 6 h resulted in a marked and rapid loss of cell viability after reoxygenation. Accordingly, we set the anoxic time to 5 h, and determined cell viability serially for 2 h after reoxygenation. As shown in Figure 1, anoxia/reoxygenation decreased hepatocyte viability linearly, though the viability of normoxic controls was not affected during the corresponding period. Preincubation with 0-1 mmol/L H_2O_2 did not improve such anoxia/reoxygenation injury, and the highest concentration of H_2O_2 somewhat worsened cell viability.

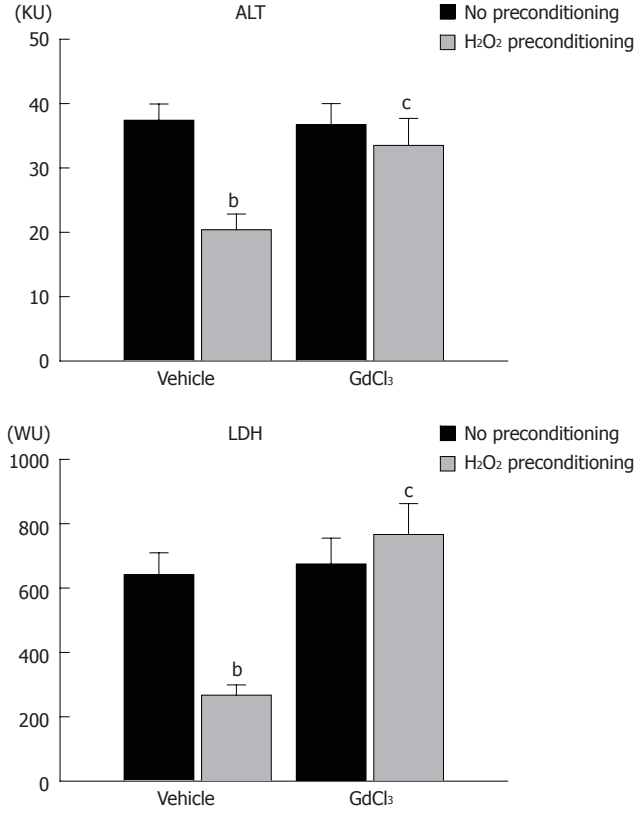


Figure 2 ALT and LDH activities in perfusate after ischemia/reperfusion in an isolated perfused liver system from no preconditioning control, H_2O_2 preconditioning, $GdCl_3$-no preconditioning, and $GdCl_3/H_2O_2$ preconditioning rats. Columns and bars represent means ± SE from six rats in each group. $^bP < 0.01$ *vs* no preconditioning, $^cP < 0.05$ *vs* vehicle treatment and H_2O_2 preconditioning. KU: Karmen unit; WU: Wróblewski unit.

Contribution of Kupffer cells to hepatocyte protection by H_2O_2 preconditioning

The results of the *in vitro* experiments suggested that H_2O_2 did not directly protect hepatocytes. We determined the contribution of Kupffer cells in H_2O_2 preconditioning, using an isolated perfused liver system. In untreated controls, which were perfused for the corresponding period without ischemia/reperfusion, ALT and LDH activity in the perfusate was 4.4 ± 0.6 and 35.1 ± 7.9, respectively (data not shown). As shown in Figure 2, warm ischemia/reperfusion markedly increased ALT and LDH activity in the perfusate to 37.4 ± 2.5 and 642 ± 68, respectively, with H_2O_2 preconditioning significantly reducing these values. $GdCl_3$ pretreatment itself did not change ALT and LDH activity after warm ischemia/reperfusion. In the H_2O_2-preconditioned group, however,

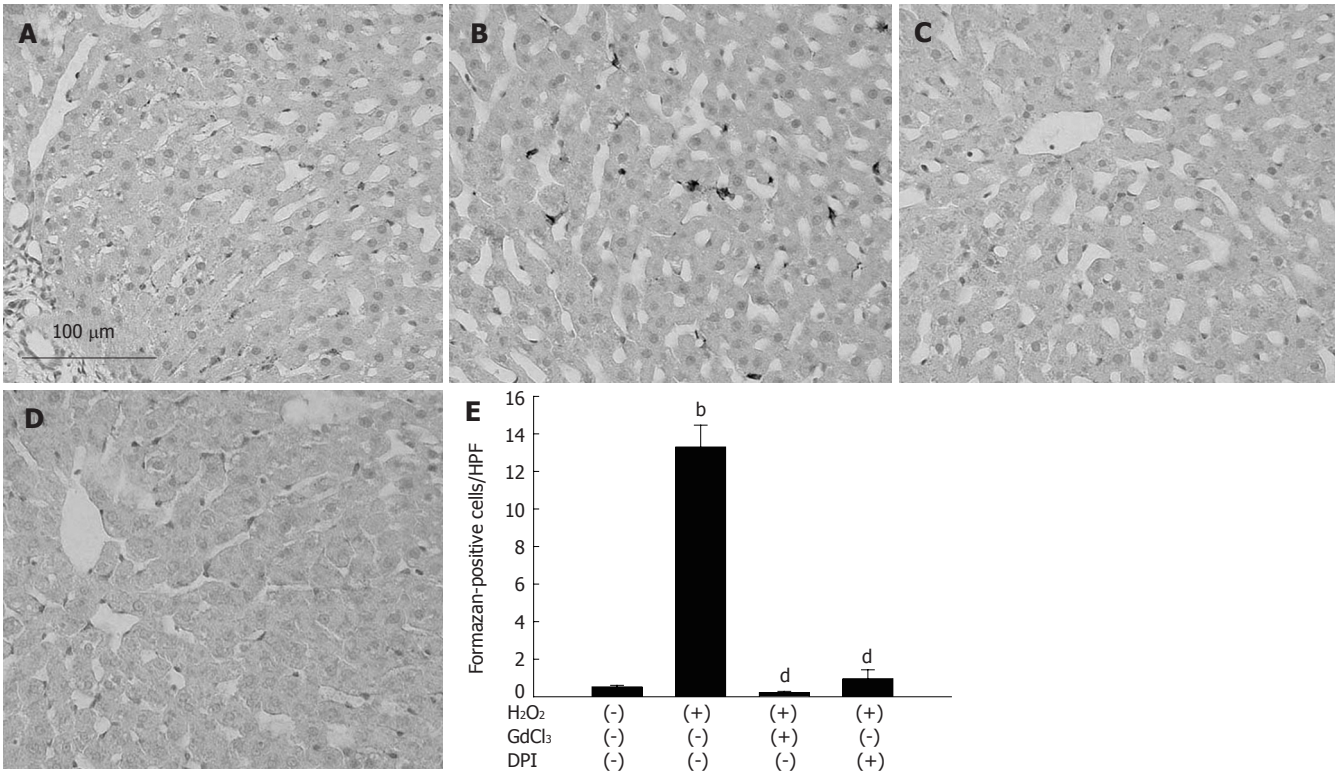


Figure 3 Oxygen radical formation in Kupffer cells after H_2O_2 preconditioning. **A**: Non-preconditioned livers; **B**: Livers after 10 min of H_2O_2 perfusion; **C**: Pretreatment with $GdCl_3$ and H_2O_2 perfusion; **D**: Treatment with DPI and H_2O_2 perfusion; **E**: The number of formazan-positive cells. Columns and bars represent means ± SE from 3 rats in each group. $^bP < 0.01$ *vs* non-preconditioned control; $^dP < 0.01$ *vs* H_2O_2 perfusion.

$GdCl_3$ pretreatment reversed the decrease in ALT and LDH activity by H_2O_2 preconditioning.

Production of oxygen radicals in Kupffer cells after H_2O_2 preconditioning

To elucidate the mechanisms of the contribution of Kupffer cells in H_2O_2 preconditioning, we determined oxygen radical production in Kupffer cells. Livers were perfused with H_2O_2 for 10 min and then perfused with NBT. When non-preconditioned livers were perfused with NBT, formazan deposition did not develop (Figure 3A). As shown in Figure 3B, H_2O_2 perfusion induced formazan deposition in non-parenchymal cells. When rats were pretreated with $GdCl_3$ injection, H_2O_2 perfusion did not induce such dense deposition (Figure 3C). Moreover, formazan deposition did not develop after H_2O_2 perfusion in livers in which DPI was added to the perfusate during H_2O_2 perfusion (Figure 3D). The number of formazan-positive cells from three rats in each group is shown in Figure 3E; treatment with $GdCl_3$ or DPI significantly reduced the number of formazan-positive cells in H_2O_2-perfused livers to the non-preconditioned control level.

Contribution of NADPH oxidase to hepatoprotection by H_2O_2 preconditioning

To determine the contribution of NADPH oxidase to the effect of H_2O_2 preconditioning, DPI was added to the perfusate during H_2O_2 preconditioning, and livers were then subjected to warm ischemia/reperfusion. We confirmed that H_2O_2 preconditioning decreased ALT and LDH activity after warm ischemia/reperfusion in a reproducible fashion. DPI treatment reversed this decrease in ALT and LDH activity induced by H_2O_2 preconditioning, although without H_2O_2 preconditioning, DPI alone did not change these values (Figure 4).

Contribution of NADPH oxidase to hepatoprotection by ischemic preconditioning

Finally, we determined whether DPI treatment could also reverse the protective effect of ischemic preconditioning. Ischemic preconditioning was performed with buffer containing DPI and livers were subjected to warm ischemia/reperfusion. As shown in Figure 5, ischemic preconditioning significantly reduced the increase in ALT and LDH activity after warm ischemia/reperfusion, with DPI reversing the hepatoprotection induced by ischemic preconditioning.

DISCUSSION

We have previously reported that brief liver perfusion with a buffer containing a low dose of H_2O_2 reduces warm ischemia/reperfusion injury similar to that with ischemic preconditioning[19]. However, the mechanisms of such H_2O_2 preconditioning have not been clarified. Since H_2O_2 is produced as a consequence of ROS metabolism, and is now considered to be involved in not only pathological, but also physiological mechanisms[25-27], it may be that H_2O_2 derived from Kupffer cells induces adaptation of hepatocytes to ischemia/reperfusion injury. Accordingly, in the present study, we sought to determine whether pretreatment with H_2O_2 directly protects

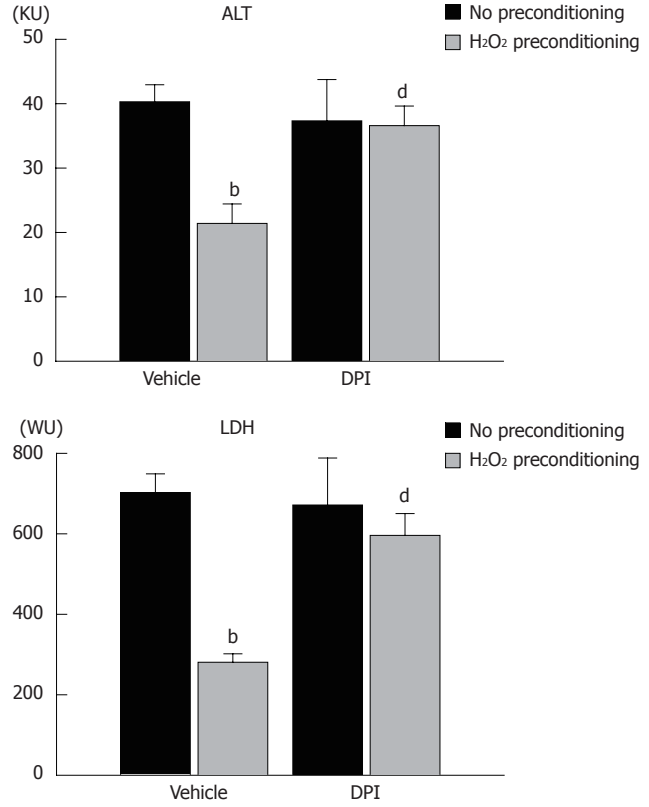


Figure 4 ALT and LDH activities in perfusate after ischemia/reperfusion in an isolated perfused liver system from no preconditioning control, H_2O_2 preconditioning, DPI-no preconditioning, and DPI/H_2O_2 preconditioning rats. Columns and bars represent means ± SE from eight rats in each group. $^bP < 0.01$ *vs* no preconditioning; $^dP < 0.01$ *vs* vehicle treatment and H_2O_2 preconditioning.

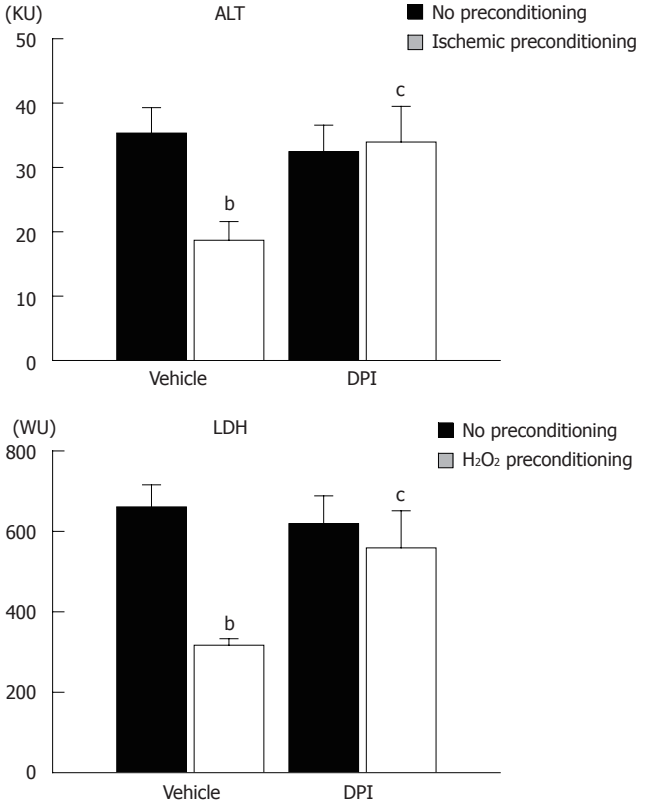


Figure 5 ALT and LDH activity in perfusate after ischemia/reperfusion in an isolated perfused liver system from no preconditioning control, ischemic preconditioning, DPI-no preconditioning, and DPI-ischemic preconditioning rats. Columns and bars represent means ± SE from six rats in each group. $^bP < 0.01$ *vs* no preconditioning; $^cP < 0.05$ *vs* vehicle treatment and ischemic preconditioning.

isolated hepatocytes against subsequent warm ischemia/reperfusion injury.

To simulate ischemia/reperfusion injury *in vitro*, isolated rat hepatocytes were incubated under anoxic conditions using an anaerobic chamber, and subsequently subjected to reoxygenation, following a widely used model[28,29]. Cell viability after reoxygenation was assessed with propidium iodide fluorometry. Propidium iodide binds to the double-stranded nucleic acids of permeable, non-viable cells[21], and the fluorescence is linearly related to LDH release[30]. Therefore, the changes in fluorescent intensity after anoxia/reoxygenation possibly represent *in vitro* ischemia/reperfusion injury. As shown in Figure 1, the viability of hepatocytes decreased slowly during anoxia and then rapidly after reoxygenation, compared with cells consistently maintained under normoxic conditions. These results were similar to those previously reported that showed necrotic hepatocytes after anoxia/reoxygenation[29]. We pretreated cells with H_2O_2 before such anoxia/reoxygenation injury; however, the viability of hepatocytes was not improved with any of the concentrations of H_2O_2 examined. Pretreatment with 1 mmol/L H_2O_2 for 10 min even worsened hepatocyte viability, although liver perfusion with the same concentration of H_2O_2 for 10 min reduced warm ischemia/reperfusion injury in our earlier study[19]. As some reports have indicated that isolated hepatocytes have lower tolerance to H_2O_2 than those *in vivo*, because of the absence of a sinusoidal structure[31-33], the present results might suggest that the effective dose of H_2O_2 in *in vitro* conditions is different from that during perfusion experiments. However, another interpretation is that sublethal H_2O_2 concentrations did not directly protect hepatocytes against warm ischemia/reperfusion injury.

Based on the hypothesis that Kupffer cells mediate hepatoprotection by H_2O_2 preconditioning, we performed liver perfusion experiments using rats in which Kupffer cell function was inhibited by $GdCl_3$ injection[22,23]. As shown in Figure 2, $GdCl_3$ alone did not change ischemia/reperfusion injury, which showed that Kupffer cells were not involved in the development of warm ischemia/reperfusion injury in our model. Accordingly, it is suggested that hepatoprotection by H_2O_2 preconditioning cannot be ascribed to the suppression of the deleterious function of Kupffer cells. Moreover, $GdCl_3$ reversed the effect of H_2O_2 preconditioning. As previously recognized, inhibition of Kupffer cell function also reversed the effect of ischemic preconditioning[19]. Therefore, we concluded that H_2O_2 preconditioning protected hepatocytes via Kupffer cells, as in ischemic preconditioning.

Kupffer cells are the principal source of oxygen radicals after prolonged hepatic ischemia/reperfusion[34]. Moreover, even after a brief period of ischemia, Kupffer cells predominantly produce oxygen radicals[19]. If H_2O_2 preconditioning protects hepatocytes via stimulation of Kupffer cells, as in ischemic preconditioning, a brief period of perfusion with H_2O_2 may induce oxygen radical

formation in Kupffer cells. As NBT reacts with oxygen radicals to form insoluble blue formazan, the NBT perfusion technique enables one to evaluate oxygen radical production *in situ*[35,36]. As shown in Figure 3, histological analysis indicated that H_2O_2 perfusion stimulated Kupffer cells to produce oxygen radicals. It has been reported that structural changes in Kupffer cells, or transient increases in the phagocytosis of Kupffer cells, is induced by perfusion with 0.7 mmol/L H_2O_2 for 10 min or 1 mmol/L H_2O_2 for 5 min, respectively[33,37]. These observations support our present findings; that stimulation of Kupffer cells with 1 mmol/L H_2O_2 for 10 min produced oxygen radicals.

Kupffer cells generate oxygen radicals by activating NADPH oxidase from a wide variety of stimulations[38-40]. However, it has never been determined whether H_2O_2 activates NADPH oxidase in Kupffer cells. Therefore, we sought to determine the contribution of NADPH oxidase to oxygen radical production in Kupffer cells by brief H_2O_2 perfusion. As shown in Figure 3, oxygen radical formation induced by H_2O_2 perfusion was eliminated by inhibition of NADPH oxidase with DPI. Some of these histological findings suggested that even brief H_2O_2 perfusion induced oxygen radical formation via NADPH oxidase in Kupffer cells. The active NADPH oxidase complex, which is bound to the plasma membrane in Kupffer cells, primarily produces superoxide anion radicals by reducing extracellular oxygen with electrons from cytosolic NADPH[40-42]. Since DPI acts on the cytosolic component of NADPH oxidase[43] and H_2O_2 permeates the cell membrane[41,44], an increase in intracellular H_2O_2 concentration by H_2O_2 treatment might act on NADPH oxidase in Kupffer cells. Subsequently, we examined the contribution of NADPH oxidase to the effect of H_2O_2 preconditioning against warm ischemia/reperfusion injury. As shown in Figure 4, inhibition of NADPH oxidase reversed the hepatoprotective effect of H_2O_2 preconditioning. Taken together with histological findings, the results indicate that NADPH oxidase in Kupffer cells mediates hepatocyte protection in H_2O_2 preconditioning.

Finally, we determined whether NADPH oxidase contributes to the effect of ischemic preconditioning. As shown in Figure 5, hepatocyte protection in ischemic preconditioning was also reversed by inhibition of NADPH oxidase. We concluded that H_2O_2 preconditioning, as well as ischemic preconditioning protects hepatocytes via NADPH oxidase in Kupffer cells. Moreover, the present results suggest that extracellular superoxide anion radicals produced by NADPH oxidase in Kupffer cells play a crucial role in hepatocyte protection by both H_2O_2 and ischemic preconditioning. Extracellular free radicals do not permeate the cell membrane[41,44] and directly oxidize the lipid bilayer of the cell membrane[45]. Thus, experiments to investigate the contribution of lipid peroxidation to hepatocyte preconditioning against ischemia/reperfusion injury are presently underway in our laboratory.

In conclusion, we report, to the best of our knowledge, a novel finding that hepatocyte protection against warm ischemia/reperfusion injury was achieved via NADPH oxidase in Kupffer cells. This was induced by preconditioning with a sublethal dose of H_2O_2 perfusion or brief ischemia/reperfusion. Based on the results of an earlier study, we assumed that a sublethal dose of H_2O_2 directly set off hepatocyte protection against ischemia/reperfusion injury. However, the present results reaffirmed the importance of Kupffer cells in induction of ischemic tolerance in liver. In general, Kupffer cells and their production of oxidative stress are considered to be a detrimental factor in hepatic ischemia/reperfusion injury[1,3]. However, under sublethal conditions, they actually could play a principal role in hepatocyte preconditioning against such injury. According to this concept, to obtain hepatoprotection, Kupffer cell activation may have to be strictly controlled between a steady-state level and a stimulated injurious level. Investigations to elucidate how sublethal oxidative stress induces these protective effects on hepatocytes are needed to determine any clinical application.

ACKNOWLEDGMENTS

Portions of this work were presented at the 56th Annual Meeting of the American Association for the Study of Liver Diseases, San Francisco, CA, United States, November 12-15, 2005.

COMMENTS

Background

As a therapeutic strategy against hepatic ischemia/reperfusion injury, which can occur in a number of clinical settings such as liver transplantation and hepatic resection, recent studies have drawn attention to ischemic preconditioning. Ischemic preconditioning is a phenomenon in which brief periods of ischemia followed by reperfusion render tissues resistant to subsequent prolonged ischemia/reperfusion. The authors have previously shown that reactive oxygen species derived from Kupffer cells mediate the hepatoprotection induced by ischemic preconditioning, and pretreatment with a sublethal dose of H_2O_2 mimics such hepatoprotection. However, the mechanism of H_2O_2 preconditioning or the role of H_2O_2 in the preconditioning phenomenon has not been determined.

Research frontiers

Clinical investigations of ischemic preconditioning in human liver resection and transplantation have recently been reviewed (*World J Gastroenterology* 2007; 13: 657-670). New findings about the beneficial role of reactive oxygen species in some experimental settings are increasingly being reported.

Innovations and breakthroughs

Cell-specific investigations of ischemic preconditioning have rarely been reported. The authors focused on Kupffer cells and their production of reactive oxygen species, which are in general regarded as detrimental factors in hepatic injury, and showed that, paradoxically, they mediated hepatoprotection induced by ischemic preconditioning. In the present article, we also demonstrated that superoxide anion radicals produced by NADPH oxidase in Kupffer cells were implicated in the hepatoprotective effects of ischemic and H_2O_2 preconditioning.

Applications

Investigations into the mechanisms of ischemic preconditioning will enable the establishment of a pharmacological preconditioning regime before liver transplantation or hepatic surgery, leading to a reduction in ischemia/reperfusion injury.

Terminology

Interruption of tissue blood flow followed by reperfusion causes tissue injury, called ischemia/reperfusion injury. Ischemic preconditioning is a phenomenon whereby brief periods of ischemia followed by reperfusion render tissues resistant to subsequent ischemia/reperfusion injury.

Peer review

This is an interesting study, well written and well designed. The methods are sound. The discussions are to the point and not overstated.

REFERENCES

1 **Lemasters JJ**, Thurman RG. Reperfusion injury after liver preservation for transplantation. *Annu Rev Pharmacol Toxicol* 1997; **37**: 327-338
2 **Serracino-Inglott F**, Habib NA, Mathie RT. Hepatic ischemia-reperfusion injury. *Am J Surg* 2001; **181**: 160-166
3 **Jaeschke H**. Molecular mechanisms of hepatic ischemia-reperfusion injury and preconditioning. *Am J Physiol Gastrointest Liver Physiol* 2003; **284**: G15-G26
4 **Banga NR**, Homer-Vanniasinkam S, Graham A, Al-Mukhtar A, White SA, Prasad KR. Ischaemic preconditioning in transplantation and major resection of the liver. *Br J Surg* 2005; **92**: 528-538
5 **Ploeg RJ**, D'Alessandro AM, Knechtle SJ, Stegall MD, Pirsch JD, Hoffmann RM, Sasaki T, Sollinger HW, Belzer FO, Kalayoglu M. Risk factors for primary dysfunction after liver transplantation--a multivariate analysis. *Transplantation* 1993; **55**: 807-813
6 **Strasberg SM**, Howard TK, Molmenti EP, Hertl M. Selecting the donor liver: risk factors for poor function after orthotopic liver transplantation. *Hepatology* 1994; **20**: 829-838
7 **Lemasters JJ**, Thurman RG. The many facets of reperfusion injury. *Gastroenterology* 1995; **108**: 1317-1320
8 **Bilzer M**, Gerbes AL. Preservation injury of the liver: mechanisms and novel therapeutic strategies. *J Hepatol* 2000; **32**: 508-515
9 **Cutrn JC**, Perrelli MG, Cavalieri B, Peralta C, Rosell Catafau J, Poli G. Microvascular dysfunction induced by reperfusion injury and protective effect of ischemic preconditioning. *Free Radic Biol Med* 2002; **33**: 1200-1208
10 **Teoh NC**, Farrell GC. Hepatic ischemia reperfusion injury: pathogenic mechanisms and basis for hepatoprotection. *J Gastroenterol Hepatol* 2003; **18**: 891-902
11 **Murry CE**, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation* 1986; **74**: 1124-1136
12 **Peralta C**, Closa D, Xaus C, Gelpí E, Roselló-Catafau J, Hotter G. Hepatic preconditioning in rats is defined by a balance of adenosine and xanthine. *Hepatology* 1998; **28**: 768-773
13 **Arai M**, Thurman RG, Lemasters JJ. Contribution of adenosine A(2) receptors and cyclic adenosine monophosphate to protective ischemic preconditioning of sinusoidal endothelial cells against Storage/Reperfusion injury in rat livers. *Hepatology* 2000; **32**: 297-302
14 **Arai M**, Thurman RG, Lemasters JJ. Ischemic preconditioning of rat livers against cold storage-reperfusion injury: role of nonparenchymal cells and the phenomenon of heterologous preconditioning. *Liver Transpl* 2001; **7**: 292-299
15 **Cleveland JC**, Raeburn C, Harken AH. Clinical applications of ischemic preconditioning: from head to toe. *Surgery* 2001; **129**: 664-667
16 **Imamura H**, Takayama T, Sugawara Y, Kokudo N, Aoki T, Kaneko J, Matsuyama Y, Sano K, Maema A, Makuuchi M. Pringle's manoeuvre in living donors. *Lancet* 2002; **360**: 2049-2050
17 **Clavien PA**, Selzner M, Rüdiger HA, Graf R, Kadry Z, Rousson V, Jochum W. A prospective randomized study in 100 consecutive patients undergoing major liver resection with versus without ischemic preconditioning. *Ann Surg* 2003; **238**: 843-850; discussion 851-852
18 **Choukèr A**, Martignoni A, Schauer R, Dugas M, Rau HG, Jauch KW, Peter K, Thiel M. Beneficial effects of ischemic preconditioning in patients undergoing hepatectomy: the role of neutrophils. *Arch Surg* 2005; **140**: 129-136
19 **Tejima K**, Arai M, Ikeda H, Tomiya T, Yanase M, Inoue Y, Nagashima K, Nishikawa T, Watanabe N, Omata M, Fujiwara K. Ischemic preconditioning protects hepatocytes via reactive oxygen species derived from Kupffer cells in rats. *Gastroenterology* 2004; **127**: 1488-1496
20 **Seglen PO**. Preparation of isolated rat liver cells. *Methods Cell Biol* 1976; **13**: 29-83
21 **Trost LC**, Lemasters JJ. A cytotoxicity assay for tumor necrosis factor employing a multiwell fluorescence scanner. *Anal Biochem* 1994; **220**: 149-153
22 **Hardonk MJ**, Dijkhuis FW, Hulstaert CE, Koudstaal J. Heterogeneity of rat liver and spleen macrophages in gadolinium chloride-induced elimination and repopulation. *J Leukoc Biol* 1992; **52**: 296-302
23 **Liu P**, McGuire GM, Fisher MA, Farhood A, Smith CW, Jaeschke H. Activation of Kupffer cells and neutrophils for reactive oxygen formation is responsible for endotoxin-enhanced liver injury after hepatic ischemia. *Shock* 1995; **3**: 56-62
24 **Hasegawa T**, Kikuyama M, Sakurai K, Kambayashi Y, Adachi M, Saniabadi AR, Kuwano H, Nakano M. Mechanism of superoxide anion production by hepatic sinusoidal endothelial cells and Kupffer cells during short-term ethanol perfusion in the rat. *Liver* 2002; **22**: 321-329
25 **Suzuki YJ**, Forman HJ, Sevanian A. Oxidants as stimulators of signal transduction. *Free Radic Biol Med* 1997; **22**: 269-285
26 **Rojkind M**, Domínguez-Rosales JA, Nieto N, Greenwel P. Role of hydrogen peroxide and oxidative stress in healing responses. *Cell Mol Life Sci* 2002; **59**: 1872-1891
27 **Maemura K**, Zheng Q, Wada T, Ozaki M, Takao S, Aikou T, Bulkley GB, Klein AS, Sun Z. Reactive oxygen species are essential mediators in antigen presentation by Kupffer cells. *Immunol Cell Biol* 2005; **83**: 336-343
28 **Carini R**, De Cesaris MG, Splendore R, Vay D, Domenicotti C, Nitti MP, Paola D, Pronzato MA, Albano E. Signal pathway involved in the development of hypoxic preconditioning in rat hepatocytes. *Hepatology* 2001; **33**: 131-139
29 **Kim JS**, Qian T, Lemasters JJ. Mitochondrial permeability transition in the switch from necrotic to apoptotic cell death in ischemic rat hepatocytes. *Gastroenterology* 2003; **124**: 494-503
30 **Nieminen AL**, Gores GJ, Bond JM, Imberti R, Herman B, Lemasters JJ. A novel cytotoxicity screening assay using a multiwell fluorescence scanner. *Toxicol Appl Pharmacol* 1992; **115**: 147-155
31 **Karbowski M**, Kurono C, Nishizawa Y, Horie Y, Soji T, Wakabayashi T. Induction of megamitochondria by some chemicals inducing oxidative stress in primary cultured rat hepatocytes. *Biochim Biophys Acta* 1997; **1349**: 242-250
32 **Halliwell B**, Clement MV, Long LH. Hydrogen peroxide in the human body. *FEBS Lett* 2000; **486**: 10-13
33 **Cogger VC**, Mross PE, Hosie MJ, Ansselin AD, McLean AJ, Le Couteur DG. The effect of acute oxidative stress on the ultrastructure of the perfused rat liver. *Pharmacol Toxicol* 2001; **89**: 306-311
34 **Jaeschke H**, Bautista AP, Spolarics Z, Spitzer JJ. Superoxide generation by Kupffer cells and priming of neutrophils during reperfusion after hepatic ischemia. *Free Radic Res Commun* 1991; **15**: 277-284
35 **Mochida S**, Ogata I, Ohta Y, Yamada S, Fujiwara K. In situ evaluation of the stimulatory state of hepatic macrophages based on their ability to produce superoxide anions in rats. *J Pathol* 1989; **158**: 67-71
36 **Mochida S**, Arai M, Ohno A, Masaki N, Ogata I, Fujiwara K. Oxidative stress in hepatocytes and stimulatory state of Kupffer cells after reperfusion differ between warm and cold ischemia in rats. *Liver* 1994; **14**: 234-240
37 **Petermann H**, Lüdicke U, Nothnagel T, Dargel R. Differential effects of exogenous and endogenously generated H2O2 on phagocytic activity and glucose release of normal and cirrhotic livers. *J Hepatol* 1998; **28**: 461-470
38 **Rymsa B**, Wang JF, de Groot H. O2-. release by activated Kupffer cells upon hypoxia-reoxygenation. *Am J Physiol* 1991; **261**: G602-G607
39 **Kono H**, Rusyn I, Uesugi T, Yamashina S, Connor HD, Dikalova A, Mason RP, Thurman RG. Diphenyleneiodonium

sulfate, an NADPH oxidase inhibitor, prevents early alcohol-induced liver injury in the rat. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G1005-G1012

40 **Cross AR**, Segal AW. The NADPH oxidase of professional phagocytes-prototype of the NOX electron transport chain systems. *Biochim Biophys Acta* 2004; **1657**: 1-22

41 **Forman HJ**, Torres M. Reactive oxygen species and cell signaling: respiratory burst in macrophage signaling. *Am J Respir Crit Care Med* 2002; **166**: S4-S8

42 **Lassègue B**, Clempus RE. Vascular NAD(P)H oxidases: specific features, expression, and regulation. *Am J Physiol Regul Integr Comp Physiol* 2003; **285**: R277-R297

43 **Cross AR**, Jones OT. The effect of the inhibitor diphenylene iodonium on the superoxide-generating system of neutrophils. Specific labelling of a component polypeptide of the oxidase. *Biochem J* 1986; **237**: 111-116

44 **Halliwell B**. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *Am J Med* 1991; **91**: 14S-22S

45 **Kühn H**, Borchert A. Regulation of enzymatic lipid peroxidation: the interplay of peroxidizing and peroxide reducing enzymes. *Free Radic Biol Med* 2002; **33**: 154-172

S- Editor Zhu LH **L- Editor** Kerr C **E- Editor** Ma WH

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5079-5089
World Journal of Gastroenterology ISSN 1007-9327

BASIC RESEARCH

Protective effects and mechanisms of Baicalin and octreotide on renal injury of rats with severe acute pancreatitis

Xi-Ping Zhang, Hua Tian, Yue-Hong Lai, Li Chen, Ling Zhang, Qi-Hui Cheng, Wei Yan, Yun Li, Qing-Yu Li, Qing He, Fei Wang

Xi-Ping Zhang, Department of General Surgery, Hangzhou First People's Hospital, Hangzhou 310006, Zhejiang Province, China
Hua Tian, Li Chen, Department of General Surgery, 2nd Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou 310009, Zhejiang Province, China
Yue-Hong Lai, Zhejiang University of Traditional Chinese Medicine, Hangzhou 310053, Zhejiang Province, China
Ling Zhang, Department of Seven Year's Clinical Medicine, Shanxi Medical University, Taiyuan 310001, Shanxi Province, China
Qi-Hui Cheng, Department of Gynecology and Obstetrics, Hangzhou First People's Hospital, Hangzhou 310006, Zhejiang Province, China
Wei Yan, Yun Li, Qing-Yu Li, Qing He, Fei Wang, Manufacturing Laboratory, Hangzhou First People's Hospital, Hangzhou 310006, Zhejiang Province, China
Supported by Technological Foundation Project of Traditional Chinese Medicine Science of Zhejiang Province, No. 2003C130 and No. 2004C142; Foundation Project for Medical Science and Technology of Zhejiang Province, No. 2003B134; Grave Foundation Project for Technology and Development of Hangzhou, No. 2003123B19; Intensive Foundation Project for Technology of Hangzhou, No. 2004Z006; Foundation Project for Medical Science and Technology of Hangzhou, No. 2003A004; and Foundation Project for Technology of Hangzhou, No. 2005224
Correspondence to: Xi-Ping Zhang, MD, Department of General Surgery, Hangzhou First People's Hospital, 261 Huansha Road, Hangzhou 310006, Zhejiang Province, China. zxp99688@vip.163.com
Telephone: +86-571-87065701 Fax: +86-571-87914773
Received: May 11, 2007 Revised: June 28, 2007

Abstract

AIM: To investigate the protective effects and mechanisms of Baicalin and octreotide on renal injury of rats with severe acute pancreatitis (SAP).

METHODS: One hundred and eighty SD rats were randomly assigned to the model group, Baicalin-treated group, octreotide-treated group and sham operation group. The mortality, plasma endotoxin level, contents of blood urea nitrogen (BUN), creatinine (CREA), phospholipase A_2 (PLA_2), nitrogen monoxide (NO), tumor necrosis factor (TNF)-α, IL-6 and endothelin-1 (ET-1) in serum, expression levels of renal Bax and Bcl-2 protein, apoptotic indexes and pathological changes of kidney were observed at 3, 6 and 12 h after operation.

RESULTS: The renal pathological changes were milder in treated group than in model group. The survival at 12 h and renal apoptotic indexes at 6 h were significantly ($P < 0.05$) higher in treated group than in model group [66.67% *vs* 100%; 0.00 (0.02)% and 0.00 (0.04)% *vs* 0.00 (0.00)%, respectively]. The serum CREA content was markedly lower in octreotide-treated group than in model group at 3 h and 6 h ($P < 0.01$, 29.200 ± 5.710 μmol/L *vs* 38.400 ± 11.344 μmol/L; $P < 0.05$, 33.533 ± 10.106 μmol/L *vs* 45.154 ± 17.435 μmol/L, respectively). The expression level of renal Bax protein was not significantly different between model group and treated groups at all time points. The expression level of renal Bcl-2 protein was lower in Baicalin-treated group than in model group at 6 h [$P < 0.001$, 0.00 (0.00) grade score *vs* 3.00 (3.00) grade score]. The Bcl-2 expression level was lower in octreotide-treated group than in model group at 6 h and 12 h [$P < 0.05$, 0.00 (0.00) grade score *vs* 3.00 (3.00) grade score; 0.00 (0.00) grade score *vs* 0.00 (1.25) grade score, respectively]. The serum NO contents were lower in treated groups than in model group at 3 h and 12 h [$P < 0.05$, 57.50 (22.50) and 52.50 (15.00) μmol/L *vs* 65.00 (7.50) μmol/L; $P < 0.01$, 57.50 (27.50) and 45.00 (12.50) μmol/L *vs* 74.10 (26.15) μmol/L, respectively]. The plasma endotoxin content and serum BUN content (at 6 h and 12 h) were lower in treated groups than in model group. The contents of IL-6, ET-1, TNF-α (at 6 h) and PLA_2 (at 6 h and 12 h) were lower in treated groups than in model group [$P < 0.001$, 3.031 (0.870) and 2.646 (1.373) pg/mL *vs* 5.437 (1.025) pg/mL; 2.882 (1.392) and 3.076 (1.205) pg/mL *vs* 6.817 (0.810) pg/mL; 2.832 (0.597) and 2.462 (1.353) pg/mL *vs* 5.356 (0.747) pg/mL; 16.226 (3.174) and 14.855 (5.747) pg/mL *vs* 25.625 (7.973) pg/mL; 18.625 (5.780) and 15.185 (1.761) pg/mL *vs* 24.725 (3.759) pg/mL; 65.10 (27.51) and 47.60 (16.50) pg/mL *vs* 92.15 (23.12) pg/mL; 67.91 ± 20.61 and 66.86 ± 22.10 U/mL, 63.13 ± 26.31 and 53.63 ± 12.28 U/mL *vs* 101.46 ± 14.67 and 105.33 ± 18.10 U/mL, respectively].

CONCLUSION: Both Baicalin and octreotide can protect the kidney of rats with severe acute pancreatitis. The therapeutic mechanisms of Baicalin and octreotide might be related to their inhibition of inflammatory mediators and induction of apoptosis. Baicalin might be a promising therapeutic tool for severe acute pancreatitis.

Key words: Severe acute pancreatitis; Baicalin; Octreotide; Renal injury; Rats; Tissue microarrays

Zhang XP, Tian H, Lai YH, Chen L, Zhang L, Cheng QH, Yan W, Li Y, Li QY, He Q, Wang F. Protective effects and mechanisms of Baicalin and octreotide on renal injury of rats with severe acute pancreatitis. *World J Gastroenterol* 2007; 13(38): 5079-5089

http://www.wjgnet.com/1007-9327/13/5079.asp

INTRODUCTION

Severe acute pancreatitis (SAP) is a fatal systemic disease featuring acute onset, serious conditions, high incidence of complications and 20%-30% of mortality mainly due to multiple organ failure at its early stage[1-4]. Octreotide has been shown to exert its therapeutic effects on SAP mainly *via* inhibiting pancreatin secretion, release of inflammatory mediators and platelet aggregation, and reducing endotoxin generation[5-8]. It is found to improve prognosis and lower mortality by enhancing the kidney protection during SAP. Baical skullcap root is an essential of "Qingyitang", a representative prescription of Traditional Chinese Medicine for SAP. Baicalin is its main effective ingredient (monomer). The *in vitro* experiment of Baicalin has confirmed it has anti-bacterial, antiviral and anti-inflammatory activities. It also can inhibit platelet aggregation and eliminate oxygen-free radicals. It was found in animal experiments that Baicalin could reduce the generation of endotoxin. In addition, Baicalein, which is the initial metabolite of Baicalin, has potent effect in inhibiting pancreatin. All these pharmacologic actions can inhibit SAP during its multiple stages[9]. It is difficult to popularize octreotide especially in remote areas with poor economy since it features high price, short half-life and inconvenient administration, while Baicalin features low price, extensive routes of administration and preparation, multiple pharmacologic actions and precise therapeutic effects.

The idea of Baicalin treatment of pancreatitis was brought forward in 1999 and validated in 2000. At the beginning, the one-time Baicalin injection *via* vena dorsalis penis or vena femoralis injection was applied, which resulted in poor therapeutic effects. Later, it was found one-time injection was inappropriate due to the short half-life of Baicalin. The expected therapeutic effects could hardly be met with one-time injection. In 2001, the intravenous drip and large dosage were applied, which resulted in sound therapeutic effects. The idea was originated from the study of the principal on pancreatitis treated by Baicalein injection. Baicalin is hydrolyzed into Baicalein. The stability, solubility and therapeutic effects of Baicalin injection are all superior to those of Baicalein injection. In this experiment, the feasibility of Baicalin treatment for SAP has been studied by comparing the protective effects and mechanisms of Baicalin and octreotide on kidneys of rats with SAP.

MATERIALS AND METHODS

Experimental animals

Clean grade healthy male Sprague-Dawley (SD) rat, weighing 250-300 g, were purchased from the Experimental Animal Center of Medical School of Zhejiang University, China.

Experimental reagents

Sodium taurocholate and sodium pentobarbital were purchased from USA Sigma Company. Octreotide was purchased from Swiss Pharmaceutical Company Novartis, and 5% Baicalin injection (China National Invention Patent Number ZL200310122673.6) was prepared by the first author at 305 mmol/L osmotic pressure. Plasma endotoxin tachypleus amebocyte lysate kit was purchased from Shanghai Yihua Medical Science and Technology Corporation (Institute of Medical Analysis, Shanghai, China); the calculation unit for content is EU/mL. Serum nitrogen monoxide (NO) was purchased from Nanjing Jiancheng Bioengineering Research Institute; the calculation units for content is μmol/L. TNF-α ELISA kit was purchased from Jingmei Bioengineering Corporation; the calculation unit for content is pg/mL (ng/L). IL-6 ELISA kit was purchased from Shanghai Shenxiong Biotech Company (China); the calculation unit for content is pg/mL (ng/L). Serum secretory phospholipase A_2 enzyme Assay ELA kit (PLA_2) was purchased from R&D system Ins; the calculation unit for content is U/mL. The serum endothelin-1 ELA kit (ET-1) was purchased from Cayman Chemical Company (Catalog Number: 583151), the calculation unit for content is ng/L (pg/mL). The Bax and Bcl-2 antibodies were purchased from Santa Cruz Company, USA. The main reagents for DNA *in situ* nick end-labeling (TUNEL) staining (Takara *In Situ* Apoptosis Detection Kit) was purchased from TaKaRa Biotechnology (Dalian) Co., Ltd. PK (protease K) was purchased from Sigma Company (USA). DAB (biphenyldiamine) was purchased from China Huamei Company, China.

Preparation of animal models

The improved Aho's method[10] was adopted to prepare 135 SAP rat models *via* retrograde injection of 35 g/L sodium taurocholate to the pancreatic duct through epidural catheter and duodenal papilla. The 135 SAP rat models were randomly assigned to the model group, Baicalin-treated group and octreotide-treated group, 45 rats in each group, while other 45 rats were assigned to the sham operation group (SO group). In sham operation group, only exploratory laparotomy (i.e., entering abdominal cavity, checking the pancreas and duodenum and then abdomen closure) was performed. Thereafter, the above-mentioned groups were randomly subdivided into 3-h, 6-h and 12-h groups, 15 rats in each group. The rats were observed at 3, 6 and 12 h after operation for: (1) Mortalities of rats in all groups followed by execution of rats and observation of gross pathological changes of kidney; (2) Kidney tissue samples were collected, fixed in accordance with relevant requirements and observed for the pathological score changes of kidney under HE staining; (3) Tissue microarray was applied to prepare the tissue microarray sections (2 mm in diameter) and immunostained using SP (streptavidin-peroxidase) method. Expressions of Bax and Bcl-2 protein in the kidney tissue were observed under light microscope, and grading was carried out based on the percentage of positive cells as follows: (-) = positive

cell count < 10%, (+) = positive cell count 10%-20%, (++) = positive cell count 20%-50%, and (+++) = positive cell count > 50%; (4) TUNEL staining technique was applied to detect apoptotic cells in the kidney and then apoptotic index was calculated as follows: Apoptotic index = apoptotic cell count/total cell count × 100%; (5) The changes in blood urea nitrogen (BUN), creatinine (CREA), phospholipase A_2 (PLA_2), nitrogen monoxide (NO), tumor necrosis factor (TNF)-α, IL-6 and endothelin-1 (ET-1) contents in blood samples obtained from the heart were determined; and (6) The correlations among these indexes were analyzed.

Procedures

Fast but water restraint was imposed on all rat groups 12 h prior to the operation. The rats were anesthetized by intra-peritoneal injection of 20 g/L sodium pentobarbital (0.25 mL/100 g), laid and fixed on table, routinely shaved, disinfected and draped. After establishing the right external jugular vein transfusion passage by using the microinfusion pump for continuous transfusion (1 mL/h per 100 g), 35 g/L sodium taurocholate was administered to prepare SAP model. To establish model control group, through median epigastrium incision, the bile-pancreatic duct and hepatic hilus common hepatic duct were confirmed, the pancreas was disclosed, the duodenal papilla inside the duodenum duct wall was identified, and then a No. 5 needle was used to drill a hole in the avascular area of mesentery. After inserting a segmental eqidural catheter into the duodenal cavity *via* the hole, the bile-pancreatic duct was inserted toward the direction of papilla in a retrograde way, a microvascular clamp was used to nip the duct end temporarily and meanwhile another microvascular clamp was used to temporarily occlude the common hepatic duct at the confluence of hepatic duct. After connecting the anesthetic tube end with the transfusion converter, 35 g/L sodium taurocholate (0.1 mL/100 g) was transfused by retrograde transfusion using the microinjection pump (made by Zhejiang University) at a speed of 0.2 mL/min. After 4 to 5 min post-injection, the microvascular clamp and epidural catheter were removed. After checking for bile leakage, the hole in the lateral wall of duodenum was sutured. The anesthetic in the abdominal cavity was absorbed up by disinfected cotton ball and then the abdomen was closed. Sham operation group received laparotomy *via* upper midline incision, turning over of the pancreas and duodenum and finally closure of the abdomen.

Dosage

In Baicalin-treated group, the animal experiments of 5% Baicalin injection have been completed including the acute toxicity test and SAP rat treatment by small, middle and large dose. The large dose (10 mg/h per 100 g) can achieve the best therapeutic effect and the dosage referred to the result of the previous preliminary experiment[10]. Ten minutes after successful modeling, Baicalin-treated group was first injected 5% Baicalin injection (10 mg/100 g) *via* the external jugular vein, followed by continuous intravenous administration (10 mg/h per 100 g) by microinfusion pump. Octreotide-treated group was first injected octreotide (0.2 μg/100 g) *via* the external jugular vein, followed by continuous intravenous transfusion (10 mg/h per 100 g) by microinfusion pump at a transfusion speed of 0.2 μg/h per 100 g. All above-mentioned dosages have been proved as effective dosages in the previous preliminary experiment[10]. Both the sham operation group and model control group were injected normal saline of equivalent volume at the corresponding time points after operation. The diameter of the drilling needle is 2.0 mm.

Statistical analysis

The values were presented as mean ± SD for normal distribution variables or median and quartile range for highly skewed variables. The significance of differences among the four groups was analyzed using Kruskal-Wallis test for highly skewed data and analysis of variance (ANOVA) for normal distribution data. Multiple comparisons were subjected to Bonfferoni correction test. Chi-square test was used to evaluate equality of frequencies for discrete variables. Correlations were tested using Spearman rank correlation coefficients. A P value less than or equal to 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS version 11.5 for windows.

RESULTS

Survival rate

The mortalities of model group were 0% (0/15), 13.33% (2/15) and 33.33% (5/15) at 3, 6 and 12 h, respectively, while those of Baicalin-treated group and octreotide-treated group were 0% at different time points, indicating a marked difference at 12 h ($P < 0.05$). The whole sham operation group survived at different time points.

Serum BUN content

Serum BUN content was markedly higher in model group and treated groups than in sham operation group at all time points ($P < 0.001$). However, the content was not significantly different between Baicalin- and octreotide-treated groups at all time points. The content was lower in Baicalin-treated group than in model group at 3 and 12 h ($P < 0.05$). The content was not different between octreotide-treated group and model group at 3 h. The content was lower in Baicalin-treated group than in model group at 6 h ($P = 0.001$), lower in octreotide-treated group than in model group ($P < 0.05$), and lower in octreotide-treated group than in model group at 12 h ($P < 0.01$) (Table 1).

Serum CREA content

The CREA content was significantly higher in model group and treated groups than in sham operation group at all time points ($P < 0.001$). However, no significant difference was found between Baicalin-treated group and model group at all time points. The content was lower in octreotide-treated group than in Baicalin-treated group at 3 h and 12 h ($P < 0.01$), and also lower in octreotide-treated group than in model group at 3 h ($P < 0.01$) and 6 h ($P < 0.05$). But no marked difference was observed between Baicalin-

Table 1 Comparison of different indexes level in blood [M (Q_R)]

		Sham operation group	Model group	Baicalin treated group	Octreotide treated group
Endotoxin	3 h	0.016 (0.005)	0.053 (0.029)	0.027 (0.005)	0.033 (0.006)
(EU/mL)	6 h	0.016 (0.010)	0.059 (0.037)	0.039 (0.019)	0.031 (0.010)
	12 h	0.014 (0.015)	0.060 (0.022)	0.034 (0.015)	0.042 (0.014)
BUN	3 h	5.310 (0.940)	12.050 (4.030)	10.530 (3.625)	9.850 (3.020)
(mmol/L)	6 h	5.500 (2.200)	17.390 (3.850)	12.220 (4.530)	13.930 (5.500)
	12 h	4.860 (1.590)	22.270 (11.375)	13.720 (4.380)	13.520 (9.810)
NO	3 h	7.500 (5.000)	65.000 (7.50)	57.500 (22.50)	52.500 (15.00)
(μmol/L)	6 h	7.500 (5.000)	62.500 (38.75)	47.500 (37.50)	57.500 (15.00)
	12 h		74.100 (26.15)	57.500 (27.50)	45.000 (12.50)
TNF-α	3 h	3.900 (3.200)	41.440 (37.72)	44.930 (45.84)	39.300 (30.60)
(pg/mL)	6 h	4.000 (1.700)	92.150 (23.12)	65.100 (27.51)	47.600 (16.50)
	12 h	5.3000 (3.000)	65.020 (26.81)	47.650 (25.52)	54.500 (41.40)
IL-6	3 h	1.846 (0.346)	5.437 (1.025)	3.031 (0.870)	2.646 (1.373)
(pg/mL)	6 h	1.743 (0.838)	6.817 (0.810)	2.882 (1.392)	3.076 (1.205)
	12 h	2.036 (0.818)	5.356 (0.747)	2.832 (0.597)	2.462 (1.353)
ET-1	3 h	15.293 (4.231)	24.745 (1.011)	19.635 (6.065)	16.827 (3.775)
(pg/mL)	6 h	16.275 (3.180)	25.625 (7.973)	16.226 (3.174)	14.855 (5.747)
	12 h	14.173 (2.556)	24.725 (3.759)	18.625 (5.780)	15.185 (1.761)

Table 2 Comparison of serum CREA content (mean ± SD, μmol/L)

Groups	3 h	6 h	12 h
Sham operation group	17.867 ± 2.890	21.467 ± 3.044	19.733 ± 3.150
Model group	38.400 ± 11.344	45.154 ± 17.435	41.500 ± 12.122
Baicalin-treated group	37.615 ± 9.483	39.867 ± 13.648	50.733 ± 29.310
Octreotide-treated group	29.200 ± 5.710	33.533 ± 10.106	33.933 ± 9.145

and octreotide-treated groups. Moreover, the content was not different between octreotide-treated group and model group at 12 h (Table 2).

Gross changes and light microscopic changes of kidney

Sham operation group: Macroscopically, the morphous of kidney was normal without swelling, with no bleeding points on surface of renal cortex. Microscopically, there were normal structure of renal glomerulus, tubule and interstitium in most rats without visible pathological change; however, swelling and blurry boundary of renal tubular epithelial cells, and stenosis of lumens were found in very few rats.

Model group: Macroscopically, there was no gross change in the kidney at 3 h; but were kidney swelling, tension of renal envelope, scattered bleeding points on surface of renal envelope, and slightly hemorrhagic urine in pelvis in severe cases at 6 h and 12 h. Microscopically, there were capillary congestion of renal glomerulus, swelling, scattered necrosis and blurry boundary of renal tubule epithelial cell, stenosis or atresia of lumens, visible protein cast (Figure 1A), interstitial edema and inflammatory cell infiltration at 3 h; and capillary congestion of renal glomerulus, swelling and scattered necrosis of epithelial cell of renal tubule (Figure 1B), interstitial edema (Figure 1C) and inflammatory cell infiltration at 6 and 12 h. The floss and red cell with eosinophilic stainings were found in renal glomerulus and homogenous or red cell cast with eosinophilic staining in renal tubule (Figure 1D). There was lamellar necrosis of epithelial cell of renal tubule in few rats.

Baicalin- and octreotide-treated groups: Macroscopically, the gross renal pathological changes were milder in Baicalin- and octreotide-treated group than in model group at 6 h and 12 h. Microscopically, there were less capillary congestion of renal glomerulus, swelling of renal tubular epithelial cell, floss and red cell with eosinophilic staining in renal capsule and inflammatory cell infiltration in treated group than in model group. Mild red cell cast was found occasionally in renal tubule of treated group. There were also renal interstitial edema and scattered necrosis of renal tubular epithelial cell in few cases. There was no visible difference between Baicalin- and octreotide-treated groups. Better therapeutic effects were achieved in octreotide-treated group.

Changes of pathological score of kidney in all groups

Pathological grading of kidney: The pathological grading of kidney was used (Table 3) and two pathologists performed the evaluation of degree of pathological changes in pancreatic tissue in double-blind fashion.

Comparison of pathological score of kidney: The score was significantly higher in model group, Baicalin- and octreotide-treated groups than in sham operation group at different time points (P <0.001). However, the score was lower in Baicalin- and octreotide-treated groups than in model group at 6 h (P <0.05). The score was lower in octreotide-treated group than in model group at 12 h (P <0.05). There was not significant different between Baicalin- and octreotide-treated groups at different time points (Table 4).

Expression of Bax protein in renal tissue

Bax-positive staining was located in the kytoplasm of renal tubular epithelial cell (Table 5 and Figure 2A-E). The expression level was not different among all groups at 12 h. The level was higher in model group and Baicalin-treated group than in sham operation group at 3 h and

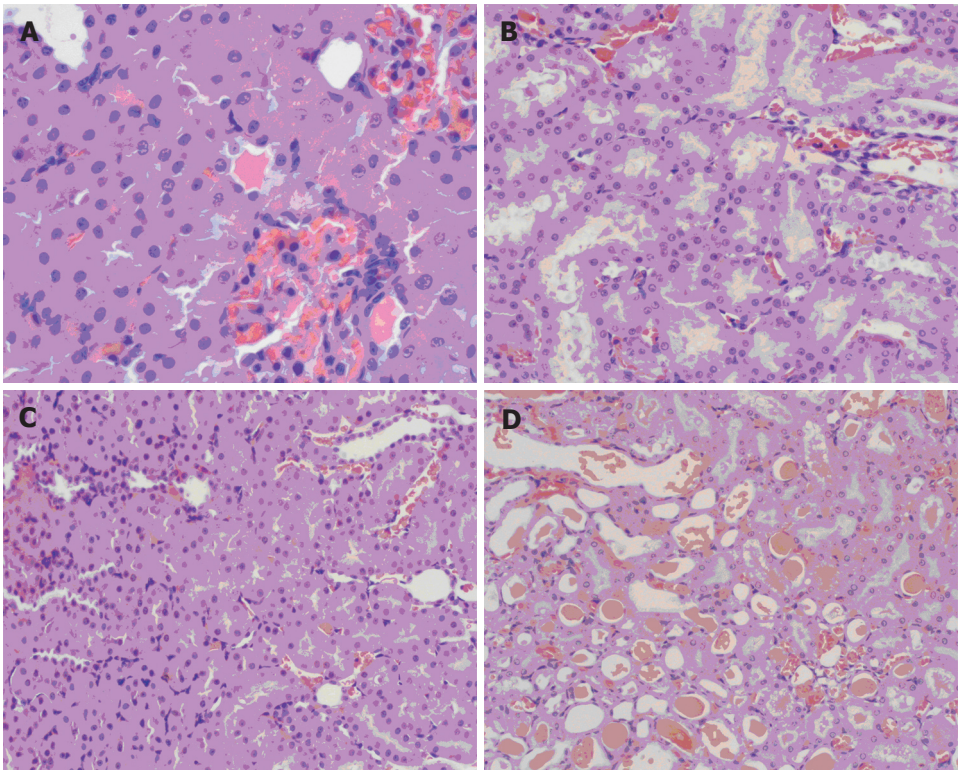


Figure 1 Light microscopic changes of kidney (HE, ×400). **A:** 3-h model group showing protein cast in renal tubule; **B** and **C:** 6-h model group showing scattered degenerative necrosis in renal tubular epithelial cells, and renal interstitial edema, respectively; **D:** 12-h model group showing visible red cell cast.

Table 3 Pathological score standard of kidney

Grade	Observation indexes
Ⅰ	No cellular proliferation or fibrosis in renal glomerulus; no capillary congestion or microthrombus; swelling and blurry boundary of renal tubular epithelial cell; stegnosis or atresia of lumens; protein cast and renal interstitial edema
Ⅱ	Glomerular capillary congestion, scattered necrosis in renal tubular epithelial cell, interstitial edema and inflammatory cell infiltration
Ⅲ	Ⅱ + lamellar necrosis of renal tubular epithelial cell

Table 4 Comparision of pathological score of kidney in all groups [$M\ (Q_R)$]

Groups	3 h	6 h	12 h
Sham operation group	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Model group	2.00 (1.00)	2.00 (1.00)	2.00 (1.25)
Baicalin treated group	1.00 (1.00)	1.00 (1.00)	2.00 (1.00)
Octreotide-treated group	1.00 (1.00)	1.00 (1.00)	1.00 (1.00)

Table 5 Expression of Bax protein in kidney

Groups		*n*	Pathologic grade			
			-	+	++	+++
Sham operation group	(3 h)	15	15			
	(6 h)	15	15			
	(12 h)	15	15			
Model group	(3 h)	15	7	4	4	
	(6 h)	13	10	1	2	
	(12 h)	10	7	1	1	1
Baicalin treated group	(3 h)	15	11	3	1	
	(6 h)	15	10	4	1	
	(12 h)	15	12	3		
Octreotide treated group	(3 h)	15	12	3		
	(6 h)	15	11	2	2	
	(12 h)	15	10	4	1	

6 h ($P < 0.05$), and also higher in octreotide-treated group than in sham operation group at 6 h ($P < 0.05$). The expression level was not different between model group and Baicalin-treated group at all time points. Similarly, the expression level was not different between Baicalin- and octreotide-treated groups. The level was lower in octreotide-treated group than in model group at 3 h ($P < 0.05$) (Table 6).

Expression of Bcl-2 protein in renal tissue

Bcl-2-positive staining was located in the kytoplasm of renal tubular epithelial cells. (Table 7 and Figure 3A-C). The level was higher in model group than in sham operation group at all time points ($P < 0.05$). The level was higher in Baicalin-treated group than in sham operation group at 12 h ($P < 0.05$), lower in octreotide-treated group than in Baicalin-treated group ($P < 0.05$), lower in Baicalin-treated group than in model group at 6 h ($P < 0.001$), and also lower in octreotide-treated group than in model group at 6 and 12 h ($P < 0.05$) (Table 8).

Comparison of renal apoptotic index

The apoptotic cells were renal tubular epithelial cells. The index was not different between model group and sham operation group at different time points. Moreover, the index was not different among all groups at 3 and 12 h. The index was higher in Baicalin- and octreotide-treated groups than in sham operation group and model group at 6 h ($P < 0.05$). The index was not different between Baicalin- and octreotide-treated groups at all time points (Table 9 and Figure 4A-D).

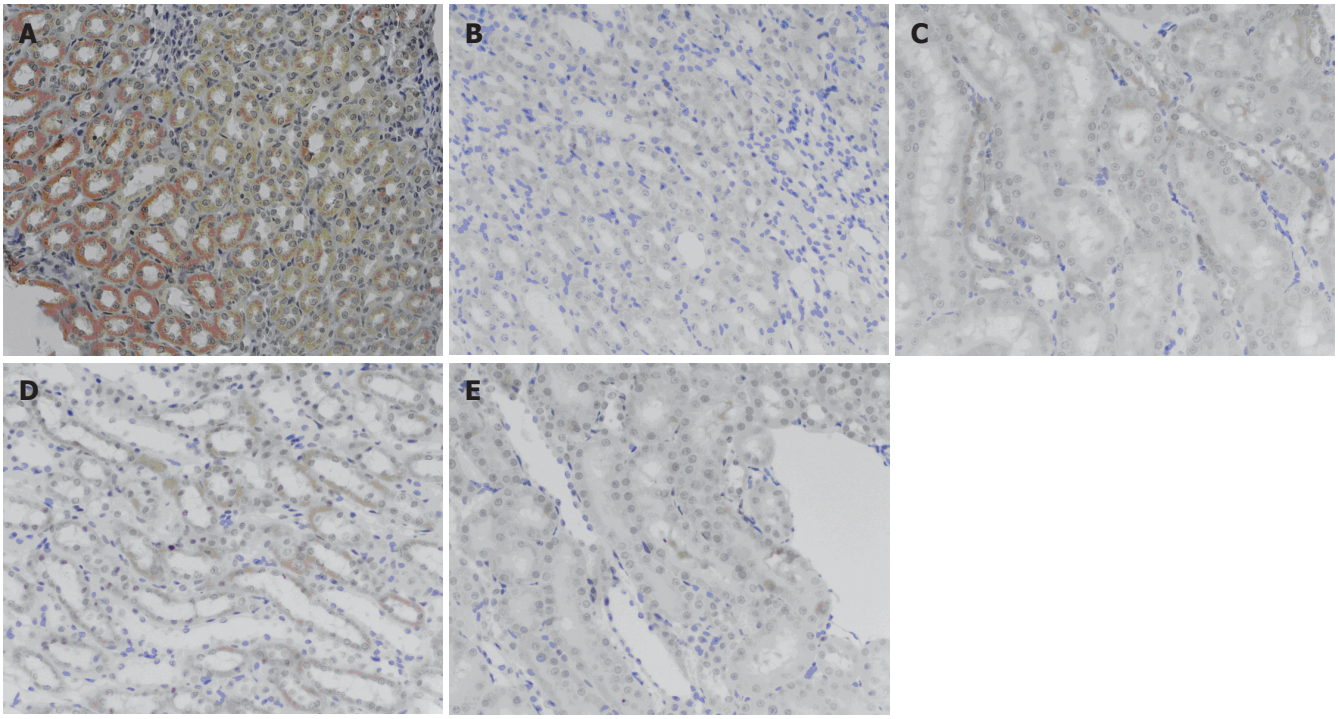


Figure 2 Bax expression in different groups (×200). **A:** 12-h model group showing high (+++) Bax expression; **B:** 3-h Baicalin-treated with negative (-) Bax expression; **C:** 12-h model group showing moderate (++) Bax expression; **D** and **E:** 6-h octreotide-treated group showing mild (+) Bax expression.

Table 6 Comparision of Bax protein in kidney [*M* (*Q*$_R$)] grade score

Groups	3 h	6 h	12 h
Sham operation group	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Model group	1.00 (2.00)	0.00 (0.50)	0.00 (1.25)
Baicalin-treated group	0.00 (0.50)	0.00 (1.00)	0.00 (0.00)
Octreotide-treated group	0.00 (0.00)	0.00 (1.00)	0.00 (1.00)

Table 7 Expression of Bcl-2 protein in kidney

Groups		*n*	Pathologic grade			
			-	+	++	+++
Sham operation group	(3 h)	15	15			
	(6 h)	15	15			
	(12 h)	15	15			
Model group	(3 h)	15	10	1	2	2
	(6 h)	13	4		1	8
	(12 h)	10	7	1	2	
Baicalin-treated group	(3 h)	15	14	1		
	(6 h)	15	15			
	(12 h)	15	11	4		
Octreotide-treated group	(3 h)	15	13	2		
	(6 h)	15	15			
	(12 h)	15	15			

Comparison of plasma endotoxin content

The content was higher in model group and treated group than in sham operation group at all time points ($P < 0.001$). The content was not different between Baicalin- and octreotide-treated groups at 6 and 12 h. The content was lower in Baicalin- and octreotide-treated groups than in model group at 3 h ($P < 0.001$), lower in Baicalin-treated group than in octreotide-treated group at 3 h ($P < 0.01$), lower in Baicalin-treated group than in model group at 6 h ($P < 0.05$), lower in octreotide-treated group than in model group at 6 h ($P = 0.001$), lower in Baicalin-treated group than in model group at 12 h ($P < 0.001$), and also lower in octreotide-treated group than in model group at 12 h ($P < 0.01$) (Table 1).

Comparison of serum PLA$_2$ content

Serum PLA$_2$ content in model group and treated groups significantly exceeded sham operation group at different time points ($P < 0.001$). At 3 h, PLA$_2$ content in Baicalin-treated group was significantly less than model group and octreotide-treated group ($P < 0.01$), but no marked difference was observed between octreotide-treated group and model group. At 6 h and 12 h, PLA$_2$ content in Baicalin- and octreotide-treated groups was significantly less than model group ($P < 0.001$). There was no marked difference between Baicalin-treated group and octreotide-treated group at 6 h, while octreotide-treated group had significantly less PLA$_2$ content than Baicalin-treated group at 12 h ($P < 0.001$) (Table 10).

Comparison of serum NO content

Serum NO content in model group, Baicalin-treated group and octreotide-treated group significantly exceeded sham operation group at different time points ($P < 0.001$). At 3 h and 12 h, Baicalin-treated and octreotide-treated groups had significantly less serum NO content than model group ($P < 0.05$). There was no marked difference in serum NO content between Baicalin-treated group and octreotide-treated group at different time points (Table 1).

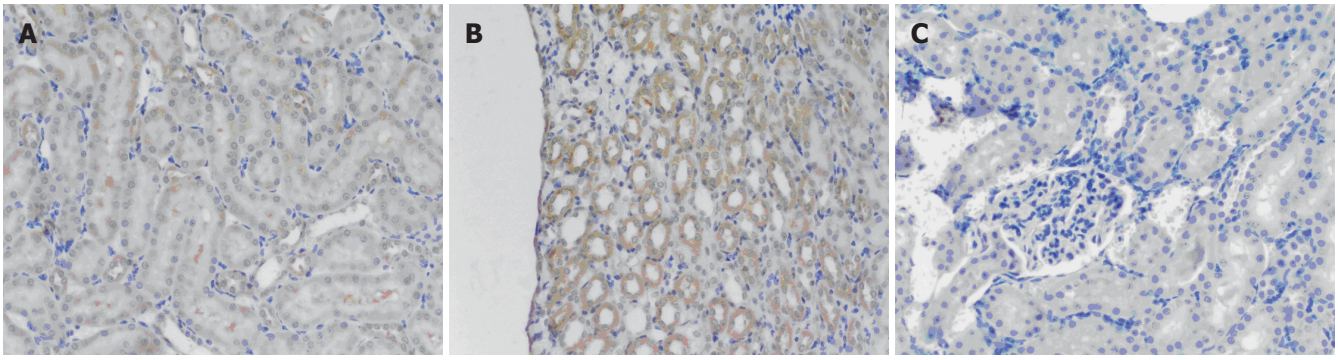


Figure 3 Bcl-2 expression in different groups (× 200). **A** and **B:** 6-h model group showing high (+++) Bcl-2 expression; **C**: 6-h octreotide-treated group with negative (-) Bcl-2 expression.

Table 8 Comparison of Bcl-2 protein in kidney [*M* (Q_R)] grade score

Groups	3 h	6 h	12 h
Sham operation group	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Model group	0.00 (2.00)	3.00 (3.00)	0.00 (1.25)
Baicalin-treated group	0.00 (0.00)	0.00 (0.00)	0.00 (1.00)
Octreotide-treated group	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

Table 9 Apoptotic index of kidney [*M* (Q_R)] (%)

Groups	3 h	6 h	12 h
Sham operation group	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Model group	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Baicalin-treated group	0.00 (0.01)	0.00 (0.02)	0.00 (0.00)
Octreotide-treated group	0.00 (0.00)	0.00 (0.04)	0.00 (0.00)

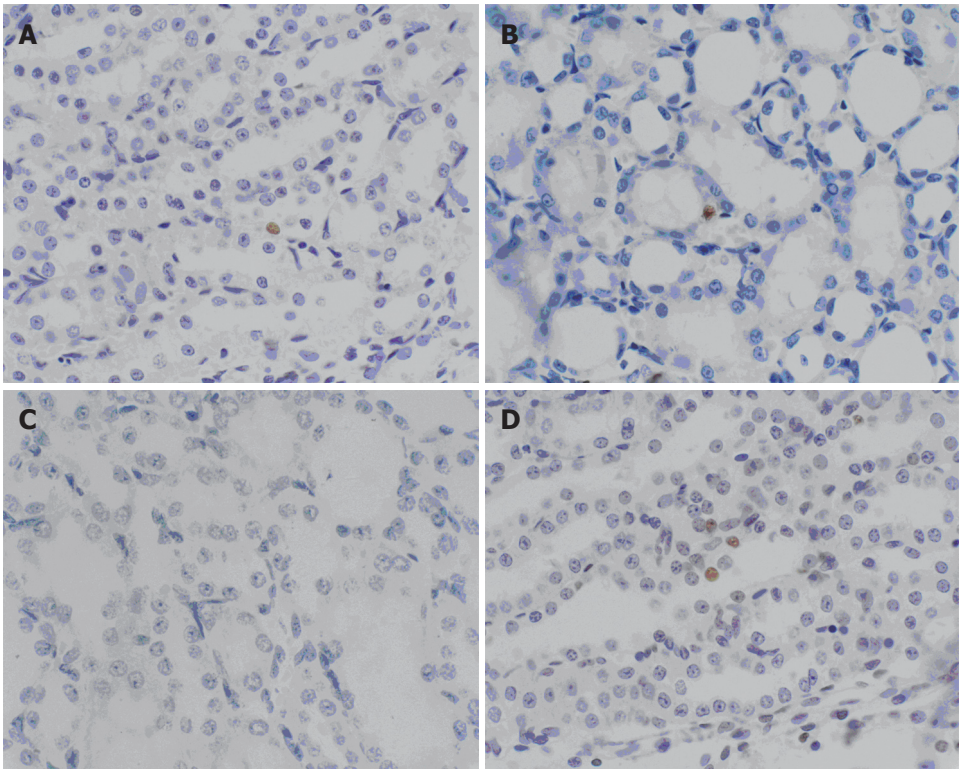


Figure 4 Apoptosis in different groups (TUNEL, × 400). **A:** 6-h octreotide-treated group showing apoptosis of renal tubular epithelial cells; **B:** 6-h Baicalin-treated group showing apoptosis of renal tubular epithelial cells; **C:** 6-h model group with no (-) apoptosis; **D:** 6-h octreotide-treated group showing apoptosis of renal tubular epithelial cells.

Comparison of serum TNF-α content

Serum TNF-α content in model group and treated groups significantly exceeded sham operation group at different time points ($P < 0.001$). There was no significant difference among model group, Baicalin-treated group and octreotide-treated group at 3 h and 12 h. At 6 h, serum TNF-α contents in Baicalin-treated group and octreotide-treated group were significantly less than model control group ($P < 0.001$); and octreotide-treated group had significantly less serum TNF-α content compared to Baicalin-treated group ($P < 0.01$) (Table 1).

Comparison of serum IL-6 content

Serum IL-6 contents at 3 h and 6 h were significantly higher in model control group and treated groups than in sham operation group ($P < 0.001$). Baicalin-treated group and octreotide-treated group had no significant difference in serum IL-6 content at all time points. Baicalin-

Table 10 Comparison of serum PLA2 content (mean ± SD, U/mL)

Groups	3 h	6 h	12 h
Sham operation group	14.62 ± 3.02	17.49 ± 3.82	19.02 ± 5.07
Model group	76.10 ± 16.70	101.46 ± 14.67	105.33 ± 18.10
Baicalin-treated group	56.25 ± 22.43	67.91 ± 20.61	66.86 ± 22.10
Octreotide-treated group	74.37 ± 19.94	63.13 ± 26.31	53.63 ± 12.28

and octreotide-treated groups had significantly lower serum IL-6 content compared to model control group at all time points ($P < 0.001$). The model control group had significantly higher serum IL-6 content than sham operation group at 12 h ($P < 0.001$), and so was Baicalin-treated group ($P < 0.01$), but no significant difference was found between octreotide-treated group and sham operation group (Table 1).

Comparison of serum ET-1 contents

Serum ET-1 content in model group was significantly higher than in sham operation group at all time points ($P < 0.001$). At all time points, Baicalin- and octreotide-treated groups had significantly lower serum ET-1 content than model group ($P < 0.001$). Octreotide-treated group had significantly lower ET-1 content compared to Baicalin-treated group at 3 h and 12 h ($P < 0.01$). At 3 h, Baicalin-treated group had significantly higher ET-1 content than sham operation group ($P < 0.01$), but no significant different was found between octreotide-treated group and sham operation group. At 6 h, there was no marked difference between Baicalin-treated group or octreotide-treated group and sham operation group, or between Baicalin-treated group and octreotide-treated group. At 12 h, octreotide-treated group and sham operation group had no marked difference, and Baicalin-treated group had significantly higher ET-1 content than sham operation group ($P < 0.001$) (Table 1).

Comparison of correlations among various indexes

Correlation between apoptotic indexes and Bax and Bcl-2 expression in the kidney: The apoptotic index of Baicalin-treated group at 3 h and that of octreotide-treated group at 3 h and 6 h was positively correlated with Bax expression ($P < 0.001$). However, there was no correlation between apoptotic index and Bcl-2 expression.

Correlation between pathological score change and BUN and CREA of kidney: The pathological score of sham operation group at 12 h was positively correlated with BUN ($P < 0.01$) and CREA levels ($P < 0.05$). The pathological score of model group was positively correlated with CREA at 3 h ($P = 0.01$) and 6 h ($P < 0.001$). The score of Baicalin-treated group at 3 h and 6 h was positively correlated with CREA ($P < 0.05$). The pathological score of Baicalin-treated group was positively correlated with BUN at 6 h ($P < 0.05$) and 12 h ($P < 0.01$). The pathological score of octreotide-treated group at 3 h was positively correlated with BUN and CREA ($P < 0.05$), and that at 6 h and 12 h was positively correlated with CREA ($P < 0.01$) and BUN ($P < 0.01$), respectively.

Correlation analysis among inflammatory mediators: PLA_2 content at 12 h was positively correlated with TNF-α in model group ($P < 0.05$), and TNF-α was positively correlated with PLA_2 content ($P < 0.01$) at 3 h.

DISCUSSION

This study demonstrated that there were milder renal pathological changes and lower serum BUN content in treated groups as compared with model group. The survival rate was higher in treated groups compared to model group. All these indicate the potent therapeutic effect of Baicalin and octreotide on rats with sever acute pancreatitis. Baicalin showed superiority over octreotide in decreasing plasma endotoxin and PLA_2 content in SAP rats at 3 h, while octreotide was found to be superior to Baicalin in alleviating renal pathological changes and decreasing CREA, Bcl-2, TNF-α, PLA_2 (at 12 h) and ET-1 contents.

Regarding the mechanisms *via* which these two drugs improve renal pathological changes, we mainly hypothesized inhibition of inflammatory mediators and induction of apoptosis.

The endotoxin[11] in plasma, and PLA_2[12,13], NO[14-18], TNF-α[19-21], IL-6[22] and ET-1[23,24] in serum are all important inflammatory mediators during SAP complicated with multiple organ injury. They are important indexes of severity and prognosis of acute pancreatitis and have two important features in common: (1) Dual effects: These inflammatory mediators, especially NO and ET-1, will protect body in low concentration and injure body in high concentration; and (2) There are interactions among different inflammatory mediators. This experiment confirmed a positive correlation between PLA_2 and TNF-α. According to many studies as well as our experiment, the concentrations of these inflammatory mediators increase during SAP[25-31]. Our experiment demonstrated that almost all indexes of inflammatory mediators were lower in treated groups than in model group, while the indexes were not different between Baicalin- and octreotide-treated groups, thereby indicating that both drugs, with similar effects, could lower the concentration of inflammatory mediators, inhibit them and protect kidney.

Both necrosis and apoptosis are ways of death of injured cells[32]. In contrast to necrosis, apoptosis does not cause intense inflammatory reaction[33], while necrosis will cause systemic inflammatory response syndrome[34]. At present, a consensus has been reached on apoptosis of pancreas during SAP[35,36]. When necrosis and apoptosis coexist in pancreas and necrosis prevails, induction of pancreatic apoptosis will result in a protective effect. We believe this conclusion is also applicable to renal apoptosis, which has been demonstrated by this experiment.

This experiment clearly showed that the renal pathological changes were milder in treated group than in model group. The renal apoptotic indexes at 6 h were markedly higher in treated group than in model group. All these indicate the renal pathological changes have been alleviated after apoptosis of renal tubular epithelial cells. In addition, the renal apoptotic indexes were not different between model group and sham operation group, possibly

because apoptosis had not occurred in model group or its incidence was too low to be detected. The occurrence of apoptosis of renal cells in treated groups, however, demonstrated that both Baicalin and octreotide could induce apoptosis. But some researchers believe the pathological changes would be aggravated by renal apoptosis during SAP[37,38], which is different from our view and therefore worth discussing.

Bax and Bcl-2 are two important apoptosis-regulating factors. The homo- or heterodimerization between anti-apoptotic Bcl-2 and proapoptotic Bax plays an important role in the apoptosis regulating function of the Bcl-2-related proteins. Interestingly, in an excess of Bax, Bax/Bax homodimers predominate, which promote apoptosis, whereas an excess of Bcl-2 leads to the formation of Bcl-2/Bax heterodimers, which inhibit apoptosis. Thus, the ratio of Bcl-2 to Bax appears to be a critical determinant of a cell's threshold for undergoing apoptosis. No expression of Bcl-2 gene has been found normal pancreatic tissue[39]. In addition, there has been no report on expression of Bcl-2 gene in normal renal tissue. It was found in this experiment that the expression levels of both Bax and Bcl-2 protein had increased during SAP, possibly because the apoptosis-inducing and -inhibiting factors had been enhanced simultaneously. As a result of the conflict of the two factors, the apoptotic indexes were not different between model group and sham operation group. However, the apoptotic indexes at 6 h were higher in treated group than in sham operation group and model group, indicating that the apoptosis of renal tubular epithelial cells occurred because the apoptosis-inducing factor prevailed in treated group. It was also found that the apoptotic indexes at 3 h were positively correlated with Bax in Baicalin-treated group. The apoptotic indexes at 3 h and 6 h were positively correlated with Bax in octreotide-treated group. But there was no correlation between apoptotic indexes and Bcl-2. Thus these data indicate that Bax might have participated in the apoptosis of renal tubular epithelial cells. Compared to model group, the expression level of Bcl-2 protein was lower in Baicalin-treated group at 6 h, and in octreotide-treated group at 6 h and 12 h, there by indicating that both Baicalin and octreotide can lower the expression level of Bcl-2 protein, enhance the apoptosis-promoting effect of Bax dimmer and thus protect kidney and alleviate its pathological changes.

The traditional histopathological section technique that has been surpassed by tissue microarray (TMA) features single sample and low efficiency[40]. The TMA we adopted has advantages such as high throughput and reliable results and great potential in oncopathologcial study[41,42]. Current studies are also mainly focused in this field[43-50]. To our knowledge, this is the first report on the application of TMA to the pathological examination of pancreatitis around the world.

In conclusion, both Baicalin and octreotide can alleviate the renal pathological changes and improve the survival of SAP rats by inhibiting inflammatory mediators, decreasing the expression level of Bcl-2 protein and enhancing the apoptosis-promoting effect of Bax dimmer to induce the apoptosis of renal tubular epithelial cells. Tissue microarray is time- and energy-saving, highly efficient and well representative in the pathological examination of pancreatitis. We believe Baicalin, a low-priced new drug with precise therapeutic effects, is hopeful to play certain role in SAP treatment in future.

COMMENTS

Background

Up to now, severe acute pancreatitis (SAP) is still an acute clinical disease featuring multiple complications, high morality and difficult treatment. Recent studies found octreotide, a somatostatin analogue, could effectively treat SAP. However, octreotide is expensive, which has hindered its clinical application. Therefore, an important direction of the current study is to find other cheap and effective drugs. Baicalin injection (China National Invention Patent Number ZL200310122673.6) prepared by the first author would be one of the best choice to treat SAP. In this experiment, the feasibility of Baicalin treatment of SAP has been studied by comparing the protecting effects and mechanisms of Baicalin and octreotide on kidneys of rats with SAP.

Research frontiers

Both Baicalin and octreotide can alleviate inflammatory reactions by inhibiting the generation of inflammatory mediators and inducing renal cell apoptosis, and thereby exert therapeutic effects on SAP. The mechanism of Baicalin- and octreotide-induced renal cell apoptosis may be related to regulation of Bax and Bcl-2 protein expressions. The therapeutic effects and mechanism of Baicalin on SAP rats are similar to those of octreotide.

Innovations and breakthroughs

As a cheap medicine with extensive pharmacological actions, few side effects and convenient administration, Baicalin can hopefully become a new drug for treating SAP. The application of tissue microarrays in pathological examination of SAP has several advantages, including time- and energy-saving, high efficiency and good representativeness, and therefore is worth popularizing.

Applications

Both Baicalin and octreotide can alleviate the renal pathological changes and improve the survival of SAP rats by inhibiting inflammatory mediators, regulating the expression level of Bax and Bcl-2 protein and inducing the apoptosis of renal tubular epithelial cell. Tissue microarray is time- and energy-saving, highly efficient and well representative in the pathological examination of pancreatitis. We believe Baicalin, a low-priced new drug with precise therapeutic effects, is hopeful to play certain role in future SAP treatment.

Terminology

Baicalin is an important monomer of Baical skullcap root. Severe acute pancreatitis (SAP) is a fatal systemic disease featuring acute onset, serious conditions, high incidence of complications and 20%-30% of mortality.

Peer review

The authors analyzed the protecting effects and mechanism of Baical skullcap root in the treatment of SAP in rats. The authors showed that Baicalin seems to be equally effective as octreotide in terms of reduction of renal pathological alterations. This study is well performed and the results merit further investigation.

REFERENCES

1 **Zhang Q**, Ni Q, Cai D. Somastostatin and growth hormone in preventing liver damage due to acute necrotizing pancreatitis *Zhonghua Yixue Zazhi* 1998; **78**: 621-623
2 **Morel DR**, Frossard JL, Cikirikcioglu B, Tapponnier M, Pastor CM. Time course of lung injury in rat acute pancreatitis. *Intensive Care Med* 2006; **32**: 1872-1880
3 **Rau BM**, Bothe A, Kron M, Beger HG. Role of early multisystem organ failure as major risk factor for pancreatic infections and death in severe acute pancreatitis. *Clin Gastroenterol Hepatol* 2006; **4**: 1053-1061
4 **Zhang Q**, Ni Q, Cai D, Zhang Y, Zhang N, Hou L. Mechanisms of multiple organ damages in acute necrotizing pancreatitis.

Chin Med J (Engl) 2001; **114**: 738-742
5 **Shor NA**, Levina VP, Ioffe IV, Andreeva IV, Chumak IuF, Zhadanov VI, Zelenyĭ II. Application of octreotide in patients with acute pancreatitis *Klin Khir* 2004; 15-17
6 **Zhu Q**, Yuan Y, Xia L, Xu J. The effect of sandostatin on sphincter of Oddi in acute severe pancreatitis in dogs *Zhonghua Neike Zazhi* 1999; **38**: 747-749
7 **Paran H**, Mayo A, Paran D, Neufeld D, Shwartz I, Zissin R, Singer P, Kaplan O, Skornik Y, Freund U. Octreotide treatment in patients with severe acute pancreatitis. *Dig Dis Sci* 2000; **45**: 2247-2251
8 **Zhang XP**, Li ZF, Liu XG, Wu YT, Wang JX, Wang KM, Zhou YF. Effects of emodin and baicalein on rats with severe acute pancreatitis. *World J Gastroenterol* 2005; **11**: 2095-2100
9 **Zhang XP**, Tian H, Cheng QH. The current situation in pharmacological study on bacalin. *Zhongguo Yaolixue Tongbao* 2003; **19**: 1212-1216
10 **Zhang XP**, Zhang L, He JX, Zhang RP, Cheng QH, Zhou YF, Lu B. Experimental study of therapeutic efficacy of Baicalin in rats with severe acute pancreatitis. *World J Gastroenterol* 2007; **13**: 717-724
11 **Ding SP**, Li JC, Jin C. A mouse model of severe acute pancreatitis induced with caerulein and lipopolysaccharide. *World J Gastroenterol* 2003; **9**: 584-589
12 **Mirković D**. The role of phospholipase A2 in the pathogenesis of respiratory damage in hemorrhagic necrotizing pancreatitis--assessment of a new experimental model. *Vojnosanit Pregl* 2000; **57**: 625-633
13 **Nevalainen TJ**, Haapamäki MM, Grönroos JM. Roles of secretory phospholipases A(2) in inflammatory diseases and trauma. *Biochim Biophys Acta* 2000; **1488**: 83-90
14 **Sun ZX**, Sun JB. The two-side biological effect of nitric oxide in acute pancreatitis. *Shoudu Yike Daxue Xuebao* 2001; **22**: 282-288
15 **Li GF**, Li Y, Li XL, Wang YH, Xu YF. Actions of nitric oxide in intestinal tract damaging following acute hemorrhagic necrotizing pancreatitis. *Dalian Yike Daxue Xuebao* 2000; **22**: 171-174
16 **Cheng S**, Zhao J, He SG, Song MM, Li ZH, Zhang YW. The role of nitric oxide in lung injury associated with acute necrotizing pancreatitis. *Zhonghua Waike Zazhi* 2003; **41**: 336-339
17 **Ren XB**, He ZP, Wen L. Effect of nitric oxide on the pancreas and kidney organ damage in rats with acute hemorrhagic necrotizing pancreatitis. *Disan Junyi Daxue Xuebao* 2001; **23**: 1081-1083
18 **Tomé LA**, Yu L, de Castro I, Campos SB, Seguro AC. Beneficial and harmful effects of L-arginine on renal ischaemia. *Nephrol Dial Transplant* 1999; **14**: 1139-1145
19 **Liu ML**, Cao WX, Tang YQ,Zhu QM. Serum tumor necrosis factor,interleukin 6,creactive protein in assessment of severity of acute pancreatitis. *Zhongguo Puwai Linchuang Yu Jichu Zazhi* 1998; **5**: 352
20 **Gong L,** Xu M. Correlation of cytokines IL-6, IL-8 and TNF-β levels in peripheral blood with APACHE 1 score in acute pancreatitis. *Zhongguo Yixian Bingxue Zazhi* 2003; **3**: 23-25
21 **Samuilov VD**, Oleskin AV, Lagunova EM. Programmed cell death. *Biochemistry* (Mosc) 2000; **65**: 873-887
22 **Yao WY**, Zhu Q, Yan YZ. Tumor Necrosis factor receptor-p55/-p75 gene expression in peripheral blood mononuclear cells in acute pancreatitis in rats. *Weichangbingxue Zazhi* 2003; **8**: 247-248
23 **Yin BB**, Ma BJ, Cai D, Ren HM, Zhang YL. Effect of endothelin in the progress of brain damage on rats of severe acute pancreatitis. *Waike Lilun Yu Shijian Zazhi* 2005; **10**: 245-251
24 **Liu JS**,Wei XG,Fu J,Liu J, Yuan YZ,Wu YL.Study of the Relationship among endo- thelin, nitric oxide, oxgen-free radical and acute pancreatitis. *Zhongguo Yishi Zazhi* 2003; **5**: 28-29
25 **Wang C**. The efficacy of growth inhibitors in the treatment of severe acute pancreatitis and its effect on plasma endotoxin and TNF-alpha. *Zhongguo Linchuang Yixue Zazhi* 2003; **19**: 1109-1110
26 **Zhang JX**, Qu JG, Li L, Xie R, Cheng GZ. The effect of labiatae on renal injury in rats with acute necrotizing pancreatitis. *Zhonghua Jizhen Yixue Zazhi* 2003; **12**: 97-102
27 **Wang DH**, Zhang XC. An Experimental study on relationship between cytokines and the translocation of intestinal bacteria and endotoxin in severe acute pancreatitis rats. *Zhongguo Yishi Zazhi* 2004; **6**: 1051-1054
28 **Yang YL**, Li JP, Li KZ, Dou KF. Tumor necrosis factor alpha antibody prevents brain damage of rats with acute necrotizing pancreatitis. *World J Gastroenterol* 2004; **10**: 2898-2900
29 **Qiu F**, Lu XS, Li YX. Low molecular weight heparin therapy for severe acute pancreatitis: a prospective clinical study. *Zhonghua Putong Waike Zazhi* 2004; **13**: 721-726
30 **Tu WF**, Zhu WM, He J, Qi XP, Feng GB, Wu RP, Li JS. Effects of BN50739 on plasma levels of endotoxin and inflammatory mediators in acute severe pancreatitis in pigs. *Zhonghua Gandan Waike Zazhi* 2002; **8**: 560-563
31 **Dong R**, Wang ZF, Lv Y, Ma QJ. Treatment of severe acute pancreatitis with large dosage of dexamethsone in the earlier time. *Gandan Waike Zazhi* 2005; **13**: 58-60
32 **Hahm KB**, Kim JH, You BM, Kim YS, Cho SW, Yim H, Ahn BO, Kim WB. Induction of apoptosis with an extract of Artemisia asiatica attenuates the severity of cerulein-induced pancreatitis in rats. *Pancreas* 1998; **17**: 153-157
33 **Samuilov VD**, Oleskin AV, Lagunova EM. Programmed cell death. *Biochemistry* (Mosc) 2000; **65**: 873-887
34 **Makhija R**, Kingsnorth AN. Cytokine storm in acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2002; **9**: 401-410
35 **Pei HH**, Liu RL, Jiang CQ, Ma LL, Fang XY. Influence of emodin on pancreatitic acinar cell apoptosis in rats with acute pancreatitis. *J Bengbu Med Coll* 2005; **30**: 112-113
36 **Pei HH**, Dai W, Zhou J. Effects of somatostatin on apoptosis of pancreatitic acinar cell apoptosis in acute necrotizing pancreatitis in rats. *Guangdong Yixue Zazhi* 2004; **25**: 138-140
37 **Ueda T**, Takeyama Y, Yasuda T, Matsumura N, Sawa H, Nakajima T, Kuroda Y. Vascular endothelial growth factor increases in serum and protects against the organ injuries in severe acute pancreatitis. *J Surg Res* 2006; **134**: 223-230
38 **Ueda T**, Takeyama Y, Hori Y, Shinkai M, Takase K, Goshima M, Yamamoto M, Kuroda Y. Hepatocyte growth factor increases in injured organs and functions as an organotrophic factor in rats with experimental acute pancreatitis. *Pancreas* 2000; **20**: 84-93
39 **Krajewski S**, Krajewska M, Shabaik A, Miyashita T, Wang HG, Reed JC. Immunohistochemical determination of in vivo distribution of Bax, a dominant inhibitor of Bcl-2. *Am J Pathol* 1994; **145**: 1323-1336
40 **Kononen J**, Bubendorf L, Kallioniemi A, Bärlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* 1998; **4**: 844-847
41 **Chen Q**, Shi QL. Application of tissue microarrays technique in lymphoma study. *Zhenduan Binglixue Zazhi* 2003; **110**: 182-184
42 **Yan XC**, Duan GJ. Study on tumors by tissue microarrays (tissue chip). *Zhongliu Fangzhi Yanjiu Zazhi* 2003; **30**: 519-521
43 **Gaiser T**, Thorns C, Merz H, Noack F, Feller AC, Lange K. Gene profiling in anaplastic large-cell lymphoma-derived cell lines with cDNA expression arrays. *J Hematother Stem Cell Res* 2002; **11**: 423-428
44 **Husson H**, Carideo EG, Neuberg D, Schultze J, Munoz O, Marks PW, Donovan JW, Chillemi AC, O'Connell P, Freedman AS. Gene expression profiling of follicular lymphoma and normal germinal center B cells using cDNA arrays. *Blood* 2002; **99**: 282-289
45 **Oka T**, Yoshino T, Hayashi K, Ohara N, Nakanishi T, Yamaai Y, Hiraki A, Sogawa CA, Kondo E, Teramoto N, Takahashi K, Tsuchiyama J, Akagi T. Reduction of hematopoietic cell-specific tyrosine phosphatase SHP-1 gene expression in natural killer cell lymphoma and various types of lymphomas/leukemias : combination analysis with cDNA expression array and tissue microarray. *Am J Pathol* 2001; **159**: 1495-1505

46 **Natkunam Y**, Warnke RA, Montgomery K, Falini B, van De Rijn M. Analysis of MUM1/IRF4 protein expression using tissue microarrays and immunohistochemistry. *Mod Pathol* 2001; **14**: 686-694
47 **Florell SR,** Coffin CM, Holden JA, Zimmermann JW, Gerwels JW, Summers BK, Jones DA, Leachman SA. Preservation of RNA for functional genomic studies: a multidisciplinary tumor bank protocol. *Mod Pathol* 2001; **14**: 116-128
48 **Kipps TJ**. Advances in classification and therapy of indolent B-cell malignancies. *Semin Oncol* 2002; **29**: 98-104
49 **Manley S**, Mucci NR, De Marzo AM, Rubin MA. Relational database structure to manage high-density tissue microarray data and images for pathology studies focusing on clinical outcome: the prostate specialized program of research excellence model. *Am J Pathol* 2001; **159**: 837-843
50 **Parker RL**, Huntsman DG, Lesack DW, Cupples JB, Grant DR, Akbari M, Gilks CB. Assessment of interlaboratory variation in the immunohistochemical determination of estrogen receptor status using a breast cancer tissue microarray. *Am J Clin Pathol* 2002; **117**: 723-728

S- Editor Zhu LH **L- Editor** Kumar M **E- Editor** Li JL

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5090-5095
World Journal of Gastroenterology ISSN 1007-9327

CLINICAL RESEARCH

Modified physiological and operative score for the enumeration of mortality and morbidity risk assessment model in general surgery

Lian-An Ding, Li-Qun Sun, Shuang-Xi Chen, Lin-Lin Qu, Dong-Fang Xie

Lian-An Ding, Shuang-Xi Chen, Lin-Lin Qu, Dong-Fang Xie, Department of General Surgery, Affiliated Hospital of Medical College, Qingdao University, Qingdao 266003, Shandong Province, China
Li-Qun Sun, Department of ICU, Haici Hospital of Medical College, Qingdao University, Qingdao 266003, Shandong Province, China
Correspondence to: Professor Lian-An Ding, MD, Department of General Surgery, Affiliated Hospital of Medical College, Qingdao University, Qingdao 266003, Shandong Province, China. dlahaolq@gmail.com
Telephone: +86-532-82913050 Fax: +86-532-82911840
Received: April 10, 2007 Revised: May 12, 2007

Abstract

AIM: To establish a scoring system for predicting the incidence of postoperative complications and mortality in general surgery based on the physiological and operative severity score for the enumeration of mortality and morbidity (POSSUM), and to evaluate its efficacy.

METHODS: Eighty-four patients with postoperative complications or death and 172 patients without postoperative complications, who underwent surgery in our department during the previous 2 years, were retrospectively analyzed by logistic regression. Fifteen indexes were investigated including age, cardiovascular function, respiratory function, blood test results, endocrine function, central nervous system function, hepatic function, renal function, nutritional status, extent of operative trauma, and course of anesthesia. Modified POSSUM (M-POSSUM) was developed using significant risk factors with its efficacy evaluated.

RESULTS: The significant risk factors were found to be age, cardiovascular function, respiratory function, hepatic function, renal function, blood test results, endocrine function, nutritional status, duration of operation, intraoperative blood loss, and course of anesthesia. These factors were all included in the scoring system. There were significant differences in the scores between the patients with and without postoperative complications, between the patients died and survived with complications, and between the patients died and survived without complications. The receiver operating characteristic curves showed that the M-POSSUM could accurately predict postoperative complications and mortality.

CONCLUSION: M-POSSUM correlates well with postoperative complications and mortality, and is more accurate than POSSUM.

Key words: Physiological and operative severity score for the enumeration of mortality and morbidity; Postoperative morbidity; Mortality; Preoperative assessment; General surgery; Critical illness

Ding LA, Sun LQ, Chen SX, Qu LL, Xie DF. Modified physiological and operative score for the enumeration of mortality and morbidity risk assessment model in general surgery. *World J Gastroenterol* 2007; 13(38): 5090-5095

http://www.wjgnet.com/1007-9327/13/5090.asp

INTRODUCTION

With the progress in medical sciences, operative indications for many medical conditions are being expanded, the range of operations is growing, with operative complications increased accordingly. Assessment of the functional condition of organs and systems assists in quantifying the operative risks. Assessment methods allow us to judge the state of an illness and carry out the appropriate preventive treatment immediately, thereby decreasing operative complications and mortality. Numerous scoring systems arecurrently available, including the Glasgow coma scale (GCS), acute physiology, age and cHealth evaluation Ⅱ (APACHE Ⅱ)[1], physiological and operative severity score for the enumeration of mortality and morbidity (POSSUM)[2] and others[3-5].

These scoring systems are important tools in deciding the course of treatment. The best known and most widely used scoring systems, APACHE Ⅱ and POSSUM, have limitations when applied to high-risk general surgical patients. APACHE Ⅱ is best suited to intensive care patients, but requires 24 h of observation, and weighing tables for individual disease state. POSSUM is limited by its somewhat subjective nature and incomplete evaluation of cardiac signs. We propose the modified POSSUM (M-POSSUM) as a reasonable, practical and objective scoring system that can be used across a broad disease spectrum in general surgery.

MATERIALS AND METHODS

Patients

The study group included 84 patients with postoperative complications who underwent surgery in our department from May 2002 to May 2004, 16 of them died. The control group included 172 patients without postoperative complications who were randomly selected during the same period of time. General clinical characteristics of the patients including age and gender are illustrated in Table 1. Diagnoses in the malignancy group included colorectal cancer, gastric carcinoma, pancreatic carcinoma, ampullary carcinoma and breast cancer. Diagnoses in the benign group included gastric ulcer, bowel obstruction, cholelithiasis and hyperthyroidism. Concomitant diseases included hypertension, coronary heart disease, pneumopathy, diabetes mellitus, hepatitis and hepatic cirrhosis, cholelithiasis, anemia, cerebrovascular disease and others.

The complications and deaths were caused by common illnesses. In this study, we defined complications as outlined in the book "Clinical General Surgery-Diagnostic Analysis and Treatment Gist". Patients were divided into a group of patients with complications and a group of patients without complications. The group of patients with complications was further divided into a group of patients who survived and a group of patients who died.

Methods

We recorded independent variables such as age, sex, function of all organs and systems, results of laboratory tests and special investigations, duration of operation and volume of intraoperative blood loss. The dependent variables were "complications or not" and "death or not" designated as "0" and "1." We used logistic regression analysis to determine significant risk factors and relative risk (RR).

The indices used were determined by our study results and the prescribed targets and standards of APACHE Ⅱ and POSSUM. The remaining factors were assigned a point value score (0, 1, 2, 3, or 4). Thus a 15-factor, five-grade scoring system was developed. We compared the difference in M-POSSUM values between various groups, plotted receiver operating characteristic (ROC) curves and calculated the area under the curves to determine the accuracy of M-POSSUM in predicting perioperative and postoperative complication and mortality rates.

Statistical analysis

All information was stored in a computer database. The general clinical data were analyzed by chi-square test. Risk factors were analyzed by logistic regression analysis. Differences in quantitative data were analyzed by *t*-test. A ROC curve was used to evaluate the ability of M-POSSUM to predict postoperative complications and mortality. All these analyses were performed using the Statistical Package for Social Sciences (SPSS) version 11.5.

RESULTS

High risk factor analysis

The RR and *P* were analyzed by logistic regression (Table 2). Eleven significant variables determined were as follows: age, cardiovascular function, respiratory function, blood test results, gastrointestinal function, endocrine function, nutritional status, hepatic function, renal function, type of incision and course of anesthesia.

Table 1 General clinical profile of patients studied

Clinic data	Postoperative complications	Postoperative death	Postoperative complications
No.	84	16	172
Age (yr)	50-79	50-79	50-79
Average age	68.67 ± 12.47	59.64 ± 13.48	69.82 ± 7.98
Gender (Male/Female)	55/29	11/5	109/63
Diagnosis, *n* (%)			
Malignancy	76 (90.5)	12 (75.0)	162 (94.2)
Benign	8 (9.5)	4 (25.0)	10 (5.8)
Concomitant diseases, *n* (%)			
Yes	14 (16.7)	1 (6.3)	68 (39.5)
No	70 (83.3)	15 (93.7)	104 (60.5)

Table 2 Relative risk of all factors

Factors	Suffered complications		Died	
	RR	*P*	RR	*P*
Age (yr) > 60	0.681	0.356	0.211	0.008
> 70	2.181	0.005	0.391	0.091
> 80	1.469	0.356	0.549	0.569
Abnormal circulation system	2.074	0.014	1.092	0.869
ECG mild change	0.893	0.274	0.374	0.933
ECG ST-T change	3.817	0.031	1.928	0.418
Abnormal respiratory system	3.581	0.000	1.723	0.311
Liver function	3.438	0.000	16.007	0.000
Blood system	2.610	0.000	2.735	0.070
Renal function	4.333	0.042	15.667	0.000
Gastrointestinal diseases	15.545	0.011	5.571	0.046
Endocrine system	2.374	0.002	1.615	0.359
Nutrition	4.938	0.000	6.000	0.003
Central nervous system	1.492	0.506	3.286	0.148
Operation time > 2 h	1.097	0.096	0.028	0.112
Operation time > 4 h	3.541	0.013	2.549	0.093
Operative hemorrhage > 300 mL	0.783	0.306	0.481	0.216
Operative hemorrhage > 500 mL	2.347	0.007	3.392	0.027
Palliative excision of malignant tumor	0.693	0.047	2.014	0.082
Malignant tumor can't excise	7.139	0.000	5.175	0.000
BP < 90/60 mmHg during operation	5.429	0.000	13.105	0.000
Steadyperioperative ECG monitoring	7.781	0.000	16.001	0.000

Development of M-POSSUM

Using our study results as well as the prescribed targets and standards of APACHE Ⅱ and POSSUM, we were able to overcome the shortcomings of APACHE Ⅱ and POSSUM, eliminate some non-significant variables, and determine the indices to be used. The remaining factors were assigned a point value score (0, 1, 2, 3 or 4). Thus, a 15-factor, five-grade scoring system was developed (Table 3).

Evaluation of M-POSSUM

We compared the M-POSSUM scores of the groups (Table 4), which were significantly different in all groups. The frequency distributional graph of M-POSSUM is shown in Figure 1. The ROC curves and areas under them are shown in Figures 2A and 2B, and Table 5. We were able to use them to predict the morbidity and mortality rates in postoperative patients and determine the accuracy of M-POSSUM in predicting morbidity and mortality.

Table 3 M-POSSUM system

Index	0	1	2	3	4
Age (yr)	< 60	60-69	70-79	⩾ 80	
Circulatory system	Normal Car Fun, BP, ECG	Car fun grade I, mild HP and abnormal ECG, sinoatrial bradycardia/tachycardia, low valtage of limb lead, BBB	Car fun grade Ⅱ, Mod. HP, well controlled by med, occasional atrial premature beats	Car fun grade Ⅲ, myocardial infarct < 3 mon, mod HP by med, ectopic, arythm, ST-T change, artrial fibrillation	Serious car, insuf, AHF, mal HP
Respiratory system	Normal	Long history of smoking, CB, asthma, URI, thick pulmonary markings	Mild COPD, mild PF change, mild pneumo	Mod COPD, Mod to serious abnormal PF	Respiratory failure
Liver function	Normal	History of hepatitis/cirrhosis of liver, TB < 34.2 μmol/L	TB 34.2-51.3 μmol/L	TB > 51.3 μmol/L	
Renal function	Normal	BUN ⩽ 10.1 mmol/L, Cr ⩽ 170 μmol/L	BUN 10.1-15 mmol/L, Cr 170-300 μmol/L	Renal failure need dialysis	
Gastrointestinal tract	Normal	History of chronic gastroenteritis, controlled peptic ulcer	Active gastrointestinal diseases(hemorrhage/ perforation of ulcers, active Crohn's disease	Percutaneous intestinal fistula	Short bowel syndrome, transplantation of small bowels
Blood system	Normal	PLT/WBC decreased mildly, Hb (rectified) > 85 g/L	Hematopathy as stable leukemia, WBC ⩾ 14.5×10^9/L	Aplastic anemia, hypersplenism syndrome, leukemia *etc.*	
Endocrine system	Normal	Mild increased BG, UGLu (-), treated hyperthyroidism, hypothyroidism, acromegaly, gout, rheumatoid disease	Mild increased BG, UGLu (+ - + +), and controlled DM, by oral medicine, hormonotherapy, active gout	DM, astable with oral medicine	Diabetic nephropathy
Nutritional Status[2]	Normal	Slight malnutrition (albumin 30-35 g/L, weight decrease < 2.5 Kg/m)	Moderate malnutrition (albumin < 30 g/L, mass decrease 2.5-5 kg/m); Radiotherapy/chemotherapy	Cachexia	
GCS	15	12-14	9-11	⩽ 8	
Operative wound		Mino (OPT < 2 h/ hemorrhage volume < 300 mL)	Mod (OPT 2-4 h/hemorrhage volume 300-500 mL)	Major (OPT > 4 h/hemorrhage volume > 500 mL)/Palliative excision of Mal tumor	M Major^{++} (excision > 3 organs) /Mal tumor can't excise
Anesthesia course				Arrhythmia/low BP < 1/2 h	continual low BP/ cardio-pulmonary resuscitation

Nor: Normal; Car: Cardiac; Fun: Function; BP: Blood pressure; HP: Hypertension; BBB: Bundle branch block; ECG: Electrocardiagram; Insuf: Insufficiency; AHF: Acute heart failure; Mod: Moderate; Med: Medicine; Mon: Month; CB: Chronic bronchitis; URI: Upper respiratory infection; PF: Pulmonary function; TB: Total bilirubin; BG: Blood glucose; UGLu: Urine glucose; DM: Diabetes mellitus; OPT: Operation time; Hm: Hemorrhage; Mal: Malignant; GCS: Glasgow coma score. [1]Liver function consul bilirubin in Child-Pough; [2]Nutritional status consider albumin.

Table 4 M-POSSUM in all groups of patients

Group	*n*	M-POSSUM	*t*-test	
			t	*P*
No complication	172	6.51 ± 2.22	-13.723	0.000
Complication	84	11.04 ± 2.95		
Alive	240	7.58 ± 2.83	-9.096	0.000
Dead	16	14.25 ± 3.02		
Alive with complication	68	10.28 ± 2.38	-5.693	0.000

Predictive formula

Logistic regression analysis yielded statistically significant equations for both morbidity and mortality. The morbidity equation was lnR/1-R = -7.287 + 0.765M-POSSUM (P = 0.000), and the mortality equation was lnR/1-R = -10.000 + 0.681M-POSSUM (P = 0.000). The predictive accuracy of morbidity equation and mortality equation was 83.6% and 94.1%, respectively.

DISCUSSION

In general surgery, postoperative morbidity rate ranges 15%-40%. Among patients at the age of 80 years or more undergoing abdominal surgery, the morbidity is higher and the mortality is about 4%[6,7]. While the range and types of operation continue to expand and the treatment guidelines for carcinoma change (progressively emphasizing radical treatment, safety and function), postoperative morbidity and mortality are increasing. Scoring systems are used to objectively assess and quantify the severity of illness, determine prognosis, guide patient monitoring and treatment, and avoid preventable deaths. Ideally, an effective scoring system should also be able to provide useful comparisons between surgeons, surgical units, hospitals and regions.

In general surgery, patients at a higher risk are mostly the elderly, who often suffer from concurrent diseases. Most routine blood biochemistry tests cannot exactly reflect any organ's physiological status. Tests and special investigations cannot replace detailed case histories and overall somatoscopy. Baseline health status, previous complications and concurrent diseases can all be found in case history. The frequently used scoring systems, APACHE Ⅱ and POSSUM, have some limitations[8-13]. They lay stress on physiological criteria, but not on hepatic function,

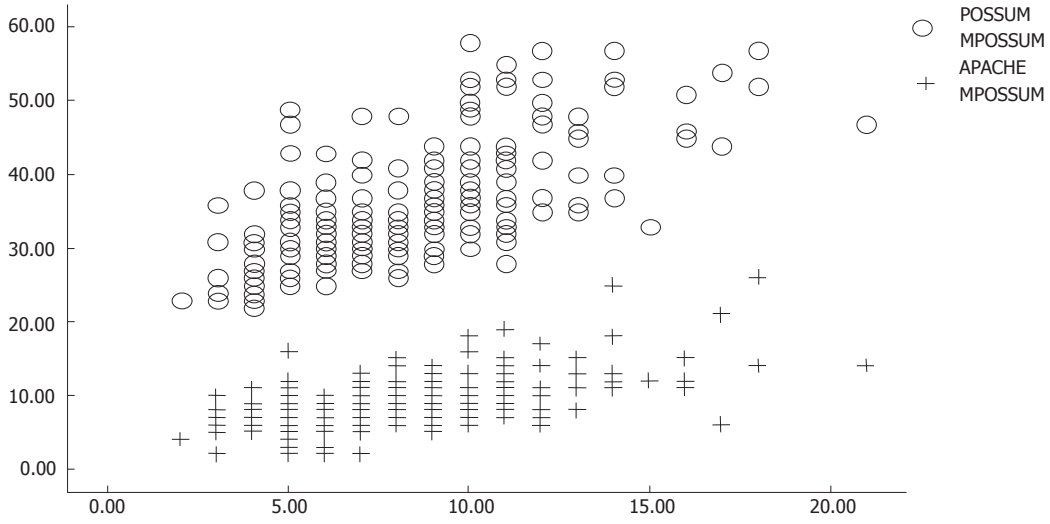


Figure 1 Relative spot chart of M-POSSUM and POSSUM with APACHE Ⅱ.

Table 5 Area under ROC curves predicting morbidity and mortality

Variaty	Area	SD	*P*
M-POSSUM (predicting for morbidity)	0.901	0.020	0.000
M-POSSUM (predicting for mortality)	0.955	0.016	0.000

blood glucose level and nutritional status, all of which were found to be independent factors affecting morbidity and mortality in our study. The POSSUM has been modified to form P-POSSUM, which is considered more accurate in predicting clinical results[5,14]. However, no other reports support it[15,16].

It has been accepted that age and morbidity is correlated with mortality. Elderly patients with concurrent diseases are more likely to develop perioperative and postoperative complications. In our study, a significant difference was found in the number of concurrent diseases between the groups of patients with and without complications. The concurrent diseases associated with a higher morbidity included high blood pressure, coronary disease, chronic lung disease, diabetes and hepatic cirrhosis. This is identical with the results in previous reports. Baue[17] reported that the main causes of death after major operations are acute respiratory distress syndrome (ARDS), stress ulcer, renal failure, intra-abdominal abscess, multiple organ dysfunction syndrome (MODS) and systemic inflammatory response syndrome (SIRS). In our study, the complications in 16 patients who died were infection, fistula, and hemorrhage, leading to MODS and shock. We found that the factors with the highest correlation with death were blood pressure, electrocardiographic activity during anesthesia, hepatic function, renal function, nutritional status, gastrointestinal function and volume of intraoperative blood loss. Surgeons should therefore improve or correct such abnormalities before operation, to prevent complications, and should promptly identify and manage perioperative and postoperative complications.

A metabolically active organism is in a state of constant dynamic balance, and a static physiological index cannot reflect the dynamic changes. In our study, the significant independent factors were determined by linear multivariate discriminant analysis.

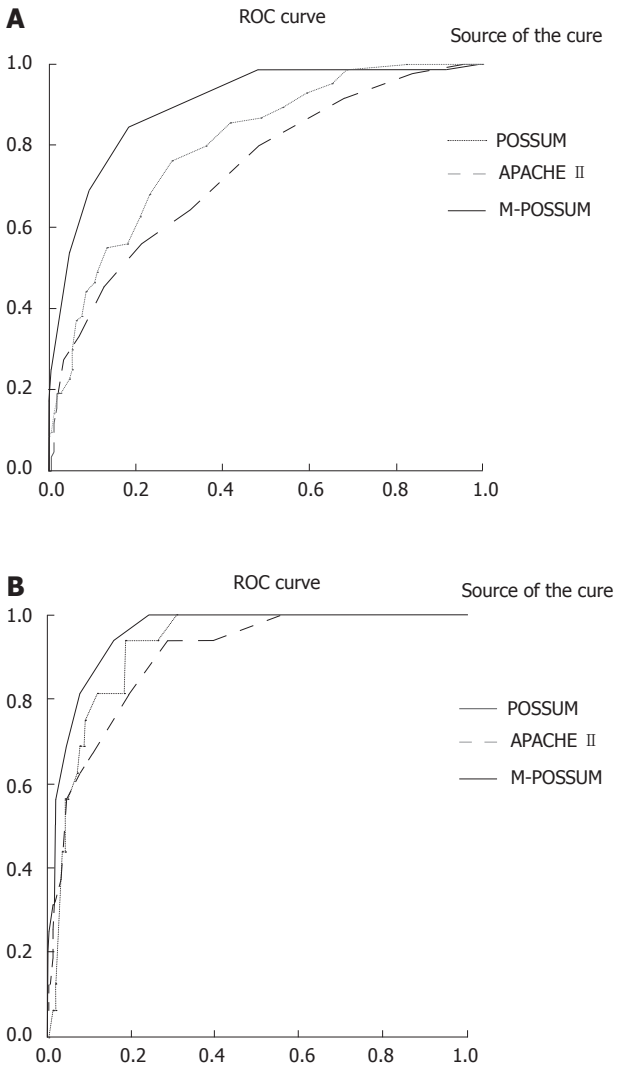


Figure 2 ROC curve of postoperative complications (**A**) and mortality (**B**). The red curve (M-POSSUM) covers the biggest area (× 100%) and illustrates the accuracy of M-POSSUM predicting the morbidity of post-operation before operation.

In the present study, the main factors influencing morbidity were gastrointestinal function, course of anesthesia, nutritional status, renal function, respiratory function, he-

patic function, cardiovascular function and a non-excisable malignant tumor. The main factors influencing mortality were hepatic function, renal function, nutritional status, digestive tract function and a non-excisable malignant tumor. These results can guide surgeons to maintain stable blood pressure and monitor electrocardiographic activity perioperatively, and to be cautious about operating on late-stage malignant tumors, in order to decrease morbidity and mortality.

In contrast to APACHE Ⅱ and POSSUM, the present scoring system is based on GCS, APACHE Ⅱ and POSSUM, supplemented with the following indices: hepatic function (bilirubin), blood glucose level, gastrointestinal function and nutritional status (albumin). However, body temperature, heart rate, sodium and potassium, which are always normal preoperatively in general surgery, are not considered. To avoid repetition, duration of operation, volume of intraoperative blood loss and surgery are considered an operative wound index for a malignant tumor. M-POSSUM also takes blood pressure and electrocardiographic activity into consideration during anesthesia.

The area under the ROC curve reflects the accuracy of prediction. In general, the accuracy is low when the area is in the range of 0.5-0.7, intermediate when the area is between 0.7 and 0.9, and high when the area is greater than 0.9. The ROC curves for morbidity and mortality demonstrate that M-POSSUM is a more accurate predictor than POSSUM. The predictive accuracy for morbidity and mortality is 83.6% and 94.1%, respectively.

Using M-POSSUM, we can obtain a numerical estimate of the health status of an individual patient prior to operation, enabling us to adjust the type and duration of operation and determine reasonably individualized postoperative monitoring and treatment so as to decrease morbidity and mortality in general surgery, especially in aged patients.

In conclusion, M-POSSUM is more accurate than POSSUM and APACHE Ⅱ in predicting postoperative morbidity and mortality, and therefore seems to be a better model for risk assessment.

ACKNOWLEDGMENTS

The authors thank Professor Zhi-Xu Wang, Medical Nutrition Institute of Qingdao University, for assistance with statistical calculation, and Director Xiu-Lin He, Nutrition Center of the Affiliated Hospital of Medical College, Qingdao University and staff of the Department of General Surgery for their help and assistance.

COMMENTS

Background

With the development in surgical techniques, the surgical domain is enlarging and more diseases are managed with surgery. The incidence of post-operation complications and mortality is increasing because of the increasing number of elderly and tumor patients. Surgeons should be able to evaluate patients effectively preoperatively, by quantitating the operative risk, in order to decrease the incidence of postoperative complications and mortality. An attempt was made to achieve this in the present study.

Research frontiers

Modern surgeons are faced with many problems, such as an aging population and complicated critical trauma. There is a significant increase in the number of patients suffering from advanced tumors and critical organ disease or dysfunction. Therefore, it is important to increase curative effects and decrease failure rates of therapy. These issues can be solved with the development of scoring systems such as APACHE Ⅰ, Ⅱ, Ⅲ and POSSUM.

Innovations and breakthroughs

Three indices of POSSUM which are poorly correlated with postoperative outcome (body temperature, serum electrolytes and type of surgery) were excluded from M-POSSUM. Six indices which are better correlated with past history, course of anesthesia, hepatic function, gastrointestinal function, endocrine function and nutritional status were included. M-POSSUM correlates well with APACHE Ⅱ and POSSUM, and is superior to both of them.

Applications

M-POSSUM can decrease postoperative complications after targeted preoperative therapeutic measures in intermediate and high risk patients.

Terminology

Scoring systems such as those defined by APACHE Ⅰ, Ⅱ, Ⅲ and POSSUM, or the American Society of Anesthesiologists (ASA) are used to quantify the severity of an illness before treatment or surgery.

Peer review

This study is interesting and scientific and readable.

REFERENCES

1 **Knaus WA**, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985; **13**: 818-829
2 **Copeland GP**, Jones D, Walters M. POSSUM: a scoring system for surgical audit. *Br J Surg* 1991; **78**: 355-360
3 **Brooks MJ**, Sutton R, Sarin S. Comparison of Surgical Risk Score, POSSUM and p-POSSUM in higher-risk surgical patients. *Br J Surg* 2005; **92**: 1288-1292
4 **Ramkumar T**, Ng V, Fowler L, Farouk R. A comparison of POSSUM, P-POSSUM and colorectal POSSUM for the prediction of postoperative mortality in patients undergoing colorectal resection. *Dis Colon Rectum* 2006; **49**: 330-335
5 **Slim K**, Panis Y, Alves A, Kwiatkowski F, Mathieu P, Mantion G. Predicting postoperative mortality in patients undergoing colorectal surgery. *World J Surg* 2006; **30**: 100-106
6 **Unalp HR**, Kamer E, Kar H, Bal A, Peskersoy M, Ali Onal M. Urgent abdominal re-explorations. *World J Emerg Surg* 2006; **1**: 10
7 **Martin RC**, Brennan MF, Jaques DP. Quality of complication reporting in the surgical literature. *Ann Surg* 2002; **235**: 803-813
8 **Nyström PO**, Bax R, Dellinger EP, Dominioni L, Knaus WA, Meakins JL, Ohmann C, Solomkin JS, Wacha H, Wittmann DH. Proposed definitions for diagnosis, severity scoring, stratification, and outcome for trials on intraabdominal infection. Joint Working Party of SIS North America and Europe. *World J Surg* 1990; **14**: 148-158
9 **Van Le L**, Fakhry S, Walton LA, Moore DH, Fowler WC, Rutledge R. Use of the APACHE II scoring system to determine mortality of gynecologic oncology patients in the intensive care unit. *Obstet Gynecol* 1995; **85**: 53-56
10 **Daffurn K**, Kerridge R, Hillman KM. Active management of the dying patient. *Med J Aust* 1992; **157**: 701-704
11 **Higgins TL**, McGee WT, Steingrub JS, Rapoport J, Lemeshow S, Teres D. Early indicators of prolonged intensive care unit stay: impact of illness severity, physician staffing, and pre-intensive care unit length of stay. *Crit Care Med* 2003; **31**: 45-51
12 **Tekkis PP**, Bentley AJ, Kocher HM, South LM, Trotter GA. Risk scoring in surgical patients. *Br J Surg* 1999; **86**: 1225
13 **Neary WD**, Heather BP, Earnshaw JJ. The Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity (POSSUM). *Br J Surg* 2003; **90**: 157-165
14 **Prytherch DR**, Whiteley MS, Higgins B, Weaver PC, Prout

WG, Powell SJ. POSSUM and Portsmouth POSSUM for predicting mortality. Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity. *Br J Surg* 1998; **85**: 1217-1220

15 **Organ N**, Morgan T, Venkatesh B, Purdie D. Evaluation of the P-POSSUM mortality prediction algorithm in Australian surgical intensive care unit patients. *ANZ J Surg* 2002; **72**: 735-738

16 **Markus PM**, Martell J, Leister I, Horstmann O, Brinker J, Becker H. Predicting postoperative morbidity by clinical assessment. *Br J Surg* 2005; **92**: 101-106

17 **Baue AE**, Durham R, Faist E. Systemic inflammatory response syndrome (SIRS), multiple organ dysfunction syndrome (MODS), multiple organ failure (MOF): are we winning the battle? *Shock* 1998; **10**: 79-89

S- Editor Ma N **L- Editor** Wang XL **E- Editor** Li JL

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5096-5100
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Risk factors associated with pancreatic fistula after distal pancreatectomy, which technique of pancreatic stump closure is more beneficial?

Marco Pericoli Ridolfini, Sergio Alfieri, Stavros Gourgiotis, Dario Di Miceli, Fabio Rotondi, Giuseppe Quero, Roberta Manghi, Giovanni Battista Doglietto

Marco Pericoli Ridolfini, Sergio Alfieri, Dario Di Miceli, Fabio Rotondi, Giuseppe Quero, Roberta Manghi, Giovanni Battista Doglietto, Department of Surgical Sciences, Gemelli University Hospital, Catholic University of the Sacred Heart School of Medicine, Rome, Italy
Stavros Gourgiotis, Hepatobiliary and Pancreatic Surgery Department, Royal London Hospital, United Kingdom
Correspondence to: Sergio Alfieri, MD, Department of Digestive Surgery, Catholic University, L.go Agostino Gemelli 8, Rome 00168, Italy. s.alfieri@rm.unicatt.it
Telephone: +39-6-30155133 Fax: +39-6-30155133
Received: March 26, 2007 Revised: June 26, 2007

Key words: Pancreas; Distal pancreatectomy; Pancreatic fistula

Ridolfini MP, Alfieri S, Gourgiotis S, Di Miceli D, Rotondi F, Quero G, Manghi R, Doglietto GB. Risk factors associated with pancreatic fistula after distal pancreatectomy, Which technique of pancreatic stump closure is more beneficial? *World J Gastroenterol* 2007; 13(38): 5096-5100

http://www.wjgnet.com/1007-9327/13/5096.asp

Abstract

AIM: To identify risk factors related to pancreatic fistula in patients undergoing distal pancreatectomy (DP) and to determine the effectiveness of using a stapled and a sutured closed of pancreatic stump.

METHODS: Sixty-four patients underwent DP during a 10-year period. Information regarding diagnosis, operative details, and perioperative morbidity or mortality was collected. Eight risk factors were examined.

RESULTS: Indications for DP included primary pancreatic disease (n = 38, 59%) and non-pancreatic malignancy (n = 26, 41%). Postoperative mortality and morbidity rates were 1.5% and 37% respectively; one patient died due to sepsis and two patients required a reoperation due to postoperative bleeding. Pancreatic fistula was developed in 14 patients (22%); 4 of fistulas were classified as Grade A, 9 as Grade B and only 1 as Grade C. Incidence of pancreatic fistula rate was significantly associated with four risk factors: pathology, use of prophylactic octreotide therapy, concomitant splenectomy, and texture of pancreatic parenchyma. The role that technique (either stapler or suture) of pancreatic stump closure plays in the development of pancreatic leak remains unclear.

CONCLUSION: The pancreatic fistula rate after DP is 22%. This is reduced for patients with non-pancreatic malignancy, fibrotic pancreatic tissue, postoperative prophylactic octreotide therapy and concomitant splenectomy.

INTRODUCTION

Distal pancreatectomy (DP), first performed by Billroth in 1884, is defined as the resection of pancreatic tissue to the left of the superior mesenteric vessels. This procedure was infrequently performed in the past because the tumours of the pancreatic tail and body were often irresectable at the time of diagnosis and due to the general dissatisfaction with this procedure for the management of chronic pancreatitis. However, the advent and development of imaging and diagnostic techniques has increased the frequency of DP. Recently, the indications for DP include malignant and benign pancreatic diseases, non-pancreatic malignancies, chronic pancreatitis, and trauma.

Pancreatic fistula is the most common major complication after DP, ranging from 5% to 40%. Pancreatic leakage often leads to further complications, such as fluid collections or intra-abdominal abscesses, wound infections, respiratory complications, and sepsis[1]. The appropriate technique of closure the pancreatic stump is still under controversial[1,2]. The most common used techniques for closure of the transected pancreatic parenchyma and for prevention of pancreatic fluid extravasations from the residual pancreatic tissue are the hand-sewn parenchymal closure and the stapled closure.

The risk factors in the development of pancreatic fistula are also unclear. The method of pancreatic stump closure, malignancy, trauma, patient's age, concomitant splenectomy, or obesity are implicated as potentially important[3,4].

The purpose of this study was to determine possible risk factors that may be associated with the onset of pancreatic fistula after DP and to identify if the method of pancreatic stump closure plays a significant role in the development of pancreatic fistula.

Table 1 Main parameters for postoperative pancreatic fistula grading[5]

Grade	A	B	C
Clinical conditions	Well	Often well	Ill appearing/bad
Specific treatment[1]	No	Yes/No	Yes
US/CT	Negative	Negative/Positive	Positive
Persistent drainage (after 3 wk)	No	Usually yes	Yes
Reoperation	No	No	Yes
Death related to fistula	No	No	Possibly yes
Signs of infection	No	Yes	Yes
Sepsis	No	No	Yes
Readmission	No	Yes/No	Yes/No

[1]Partial (peripheral) or total parenteral nutrition, antibiotics, enteral nutrition, somatostatin analogue and/or minimal invasive drainage.

MATERIALS AND METHODS

A retrospective review of 64 patients who underwent DP from January 1996 to December 2005 at the Department of Digestive Surgery, Gemelli University Hospital of Rome, was conducted.

Patients' age, sex, indications for surgery, concomitant splenectomy, additional procedures, methods of pancreatic stump closure, and postoperative complications with a specific focus on pancreatic leaks, mortality, and duration of postoperative hospital stay were recorded. Operative details also included operating time and the texture of pancreatic parenchyma. No patient was excluded from the series.

The indications for DP included either primary pancreatic diseases or non-pancreatic malignancies. All the operations were performed by same surgical team. Division of the pancreatic parenchyma was by knife or linear stapler (GIA), while the pancreatic remnant was either closed by a linear stapler or by hand running absorbable monofilament 3-0 sutures. One open drain was positioned near the transected pancreas and it was removed when the daily fluid output was lower than 10 mL or when amylase concentration in the fluid drain was unremarkable in cases of pancreatic leaks (< 300 IU).

We used prophylactic octreotide in the last 34 patients (53%), postoperatively for 7 d, while in cases with fistula the octreotide was prolonged until recovery. The dose of octreotide was 0.5 mg three times a day.

Postoperative mortality and morbidity were registered for 30 d or during the total hospitalisation time, if longer. Postoperative pancreatic leaks were classified according to the international accepted definition reported by Bassi *et al*[5] for the international Study Group on Pancreatic Fistula (Table 1). Suspicion and diagnosis of fistula based on biochemical criteria included drainage of more than 10 mL of fluid in 24 h, with an amylase content of more than 3 times the serum amylase activity (> 300 IU) for more than 10 d after surgery. The amount of fluid amylase collected from the drainage tubes, as well as the serum amylase level were evaluated daily until postoperative d 10, and longer in cases with pancreatic fistula. The clinical criteria included the presence of clinical symptomatology such as fever greater than 38℃, leucocytosis with peripheral white blood cells amount more than 10 000 cells/mm^3, intra-abdominal pain or abscess, and the need of percutaneous drainage or reoperation. Intra-abdominal collections were detected by computed tomography (CT).

Oral feeding was generally started on second postoperative day in patients with insignificant amylase concentrations in the abdominal fluid collected by drainage or in patients with Grade A fistula. Conversely, patients with pancreatic fistula Grades B or C were kept with nothing by mouth and were supported with partial or total parenteral or enteral nutrition.

The following eight risk factors were analysed: age (patients older or younger than the age of 70 years old), gender, pancreatic disease or non-pancreatic malignancy, technique of pancreatic stump closure, spleenic preservation, texture of the pancreatic parenchyma (soft or fibrotic tissue), additional procedures, and postoperative use of octreotide. The texture of the pancreatic parenchyma was adequately defined by histopathology examination.

Statistical analysis

All data were reported as the mean ± standard deviation (SD) and/or median. The data were analyzed by means of SPSS 12.01 statistical package for Windows. Mann-Whitney *U* test and Chi-square test was used for group comparison and Students' *t* test to analyze normally distributed quantitative data. $P < 0.05$ was considered statistically significant.

RESULTS

All patients underwent DP for elective benign or malignant, pancreatic or non-pancreatic diseases. There were 30 males (47%) and 34 females (53%). The patients' age ranged from 42 to 84 years (median age, 72.3 years).

The indications for surgery included 38 patients (59%) with pancreatic disease and 26 patients (41%) with non-pancreatic malignancy. Forty-three patients (67%) underwent DP with splenectomy and one or more additional procedures due to primary malignancy: 17 of these patients (40%) had primary pancreatic malignancy infiltrated surrounding organs and 26 patients (60%) had non-pancreatic malignancy infiltrated pancreas. The overall number of additional procedures was 57. Spleen preserving DP was performed in 8 patients (13%); they all had benign or borderline diseases, while DP with splenectomy was performed in 13 patients (20%). The patients' demographics, indications for surgery, operative and technical factors are summarized in Table 2.

The median postoperative length of hospital stay, in patients without fistula, was 11 d (range, 6-15 d); while in patients with pancreatic fistula the median hospitalization was prolonged: 23 d (range, 16-28 d).

The postoperative mortality rate was 1.5% (one patient died due to sepsis), while the morbidity rate was 37% (n = 24). The patient with sepsis had both pancreatic and oesophago-jejunal fistula after total gastrectomy for cardiac cancer. The last 7 years no mortality rate occurred. Fourteen patients (22%) developed a pancreatic fistula; 4 of fistulas (28.6%) were classified as Grade A, 9 (64.3%)

Table 2 Patients' demographics, indications for surgery, operative and technical factors

	No. of patients (%)
Sex	
Male	30 (47)
Female	34 (53)
Indications for surgery	
Pancreatic	
Cystadenoma	19 (30)
Adenocarcinoma	11 (17)
Neuroendocrine tumour	3 (5)
Cystadenocarcinoma	3 (5)
Chronic pancreatitis	1 (1.5)
Lymphangioma	1 (1.5)
Non-pancreatic	
Gastric adenocarcinoma	16 (25)
Retroperitoneal sarcoma	4 (6)
Colonic adenocarcinoma	2 (3)
Renal carcinoma	2 (3)
Adrenal grand carcinoma	1 (1.5)
Gastrointestinal stromal tumour	1 (1.5)
Operations	
DP + splenectomy	13 (20)
Spleen preserving DP	8 (13)
DP + splenectomy + additional procedure	43 (67)
Additional procedures	
Gastrectomy	26 (46)
Colon resection	12 (21)
Adrenalectomy	10 (17)
Small intestine resection	5 (9)
Nephrectomy	4 (7)
Closure of pancreatic stump	
Stapler	29 (45)
Suture	35 (55)

Table 3 Postoperative results

	No. of patients (%)
Death	1 (1.5)
Reoperation	2 (3)
Complications	
No	40 (63)
Yes	24 (37)
Pancreatic fistula	14 (22)
Grade A	4 (28.6)
Grade B	9 (64.3)
Grade C	1 (7.1)
Intra-abdominal hemorrhage	2 (3)
Intra-abdominal abscess	3 (5)
Pulmonary	5 (8)

as Grade B and only 1 as Grade C (7.1%). Two patients (3%) required a second operation due to postoperative intra-abdominal bleeding. None of patients required a reoperation because of intra-abdominal abscess or fluid collection; these patients were treated by percutaneous drainage. Postoperative results are showed in Table 3.

Pancreatic fistula was significantly more common in patients who underwent DP for primary malignant or benign pancreatic diseases ($P = 0.04$) and in patients who did not receive postoperative prophylactic octreotide therapy ($P = 0.01$). Of 26 patients who were operated for non-pancreatic malignant disease, only 3 (11%) experienced a leak, compared with 11 (29%) of the 38 patients who were operated for pancreatic disease only. Four (12%) of 34 patients who received octreotide developed a pancreatic leak, compared with 10 (33%) of 30 patients who did not.

Table 4 Incidence of pancreatic fistula after distal pancreatectomy according to examined risk factors

		Fistula		
	Patients ($n = 64$)	No. ($n = 50$)	Yes ($n = 14$)	*P* value
Age (yr)				NS
< 70	23 (36)	17 (34)	6 (43)	
> 70	41 (64)	33 (66)	8 (57)	
Sex				NS
Male	30 (47)	23 (46)	7 (50)	
Female	34 (53)	27 (53)	7 (50)	
Pancreatic stump closure				NS
Stapler	29 (45)	22 (44)	7 (50)	
Suture	35 (55)	28 (56)	7 (50)	
Pathology				0.04
Pancreatic disease	38 (59)	27 (54)	11 (79)	
Non-pancreatic malignancy	26 (41)	23 (46)	3 (21)	
Octreotide therapy				0.01
Yes	34 (53)	30 (60)	4 (28)	
No	30 (47)	20 (40)	10 (72)	
Texture of pancreatic parenchyma				0.006
Soft	27 (42)	15 (30)	12 (86)	
Fibrotic	37 (58)	35 (70)	2 (14)	
Concomitant splenectomy				0.002
Yes	56 (87)	46 (92)	10 (71)	
No	8 (13)	4 (8)	4 (29)	
Procedures				NS
Pancreatic resection only	21 (33)	14 (28)	7 (50)	
Additional procedures	43 (67)	36 (72)	7 (50)	

NS: No significant. Values in parentheses are percentages.

The potential relationship between the texture of the pancreatic parenchyma and pancreatic leak was also evaluated, considering the remnant stump including resection margin: only 2 (5%) of the 37 patients with fibrotic pancreatic tissue experienced a leak, compared with 12 (44%) of the 27 patients with soft tissue ($P = 0.006$). Finally, we observed significant statistical difference comparing the patients who underwent concomitant splenectomy with them who did not: 10 (18%) of 56 patients with concomitant splenectomy experienced a pancreatic fistula, while 4 (50%) of 8 patients with spleen preserving procedure developed a pancreatic leakage.

The other factors such as age, gender, technique of pancreatic stump closure, and type of surgical procedures were not significantly associated with pancreatic fistula formation. The incidence of pancreatic fistula after DP according to the eight examined risk factors is summarized in Table 4.

DISCUSSION

In this report, we describe our 10-year experience with DP for pancreatic and non-pancreatic benign diseases and malignancies. Our findings identify the importance of four risk factors in the development of pancreatic fistula: fibrotic pancreatic parenchyma, non-pancreatic disease, concomitant splenectomy, and postoperative prophylactic

octreotide therapy were found to result in a statistically significant reduction in the rate of postoperative pancreatic leakage. No significant differences were found regarding to the onset of pancreatic fistula according to the rest of examined factors such as age, gender, technique of pancreatic stump closure, and additional procedures.

Mortality and morbidity after DP have significantly decreased the last decades[6]. In this study, we support that DP can be performed with low perioperative mortality (1.5%), while the incidence of pancreatic fistula, which was the most common postoperative complication, was 22%. These results are in accordance with the most authors' conclusions[3,4,7-9].

In the past, in most of published studies there was no definition of pancreatic fistula and the diagnostic criteria for this had not yet been completely established. Balcom *et al*[10] defined fistula as the drainage of more than 30 mL of amylase-rich fluid, while Balzano *et al*[4] used a strict definition of fistula: > 5 mL for five days after the fifth postoperative day. In this study, pancreatic leaks were classified according to the International Study Group on Pancreatic Fistula definition[5]. Grade A of pancreatic fistula, called transient fistula, has no clinical impact, Grade B requires a change in management or adjustment in the clinical pathway, while in Grade C, a major change in clinical management or deviation from the normal clinical pathway occurs. Our results showed that the majority of pancreatic fistulas were belonged to Grade B. These patients were supported with parenteral nutrition, the drains were maintained in place, some of them were covered by antibiotics and all were leaded to a delay in discharge. The only patient with Grade C fistula died postoperatively due to sepsis.

The appropriate closure of the pancreatic stump after DP in order to reduce postoperative complications, especially the incidence of pancreatic fistula, remains an unsolved surgical problem. A great number of surgical techniques and instruments for treating the resected pancreatic surface and preventing pancreatic fistula after DP has been proposed: hand-sewn suture[2,11], stapler[1,2,7,11], a combination of stapler and suture[2,7,11], the use of pledgetted suture[7], fibrin-glue sealing[12,13], and prolamine injection[14]. The most commonly used techniques of pancreatic remnant management are the stapler and suture closure. Stapler division of the pancreatic parenchyma has already been found that is a simple, quick, and secure method of pancreatic stump closure[1,6,15]. The study by Bassi *et al*[16] is the only randomized controlled trial that compared the two techniques. They observed that using the stapler technique had better results in comparison with the suture closure (stapler 14% *vs* hand suture 33%). Takeuchi *et al*[1] described a statistically significant reduction in fistula rate after stapler closure, Fahy *et al*[9] described suture closure as a risk factor for pancreatic fistula, while Sheehan *et al*[2] found that the suture closure of the pancreatic remnant was superior compared with the stapler closure (25% *vs* 14% respectively). Finally, Kajiyama *et al*[15] and Bilimoria *et al*[7] found no differences between the two techniques. In our study, the authors' technical approach involved stapler and suture closure of the resected pancreatic parenchyma; in 45% of the patients the pancreatic parenchyma was closured using a linear stapler (GIA), while in 55% of the patients an absorbable monofilament 3-0 suture was used. We were unable to determine the advantage of one method of pancreatic remnant closure on the development of pancreatic fistula: onset of fistula was observed in 24% of patients in stapler group, and in 20% of patients in hand suture group.

Several studies have shown that fistula formation was not related to the main pancreatic duct ligation[3,17], while others reported the opposite[6]. We ligated and closed the main pancreatic duct with sutures in all patients after DP thinking that may be helpful in reducing possible pancreatic leaks. In almost all of them, we had no difficulty to find out the pancreatic duct. However, there was no significant difference according to the incidence of pancreatic fistula in our study in comparison with the literature studies[3,4,7-9].

Many authors state that the texture of the pancreatic parenchyma seems generally to be one of the most important risk factors responsible for the increased rate of pancreatic fistula[2,9]. The fibrotic pancreatic tissue is believed to be less likely to pancreatic leakage. In accordance with the reported studies, we observed that the patients with soft pancreatic tissue had higher incidence of pancreatic leakage compared with them who had fibrotic pancreatic parenchyma (44% *vs* 5%, $P = 0.006$).

We used postoperative prophylactic octreotide treatment in 53% of the patients and 12% of them developed a pancreatic leak, compared with 33% of patients who did not receive octreotide. However, the role of this treatment still remains unclear. Two randomized trials by Lowy *et al*[18] and Yeo *et al*[19] failed to identify a decrease in the pancreatic leakage in patients underwent pancreaticoduodenectomy, while Gouillat *et al*[20] demonstrated a decreased leak rate in a randomized trial of patients who underwent pancreaticoduodenectomy. Buchler *et al*[21] reported that the use of octreotide could prevent pancreatic fistula following pancreatic resection. They showed that the incidence of pancreatic fistula was 18% in the patients received perioperative octreotide treatment and 37% in patients received placebo after pancreatic resection. Suc *et al*[22] also described the advantage of using octreotide after pancreaticoduodenectomy.

The present report also highlights the importance of the primary disease in development of pancreatic fistula after DP: the patients underwent DP for pancreatic disease only had a higher incidence of pancreatic fistula compared with them who underwent DP for non-pancreatic malignancies. There is no doubt that it represents a quite strange observation because in patients with DP due to extrapancreatic malignancies the pancreatic tissue is generally softer than them who undergo DP for pancreatic diseases and we have no scientific explanation. However, new and larger studies are needed to determine if the extrapancreatic malignancies are associated with lower risk of pancreatic fistula after DP or not. Interestingly, there are no studies in the literature, at our knowledge, that examine the primary disease as significant risk factor in the onset of pancreatic fistula. In published series, only the underlying pancreatic disease have examined

as a responsible factor for the occurrence of pancreatic leakage[2,9]. This is the first study to show that primary (pancreatic and non-pancreatic) disease is a risk factor for postoperative morbidity after DP, especially for pancreatic leakage ($P = 0.04$).

In this study, postoperative comparison suggests that the patients underwent DP with splenectomy had a significantly lower incidence of postoperative pancreatic leakage compared with patients who underwent DP with spleen preserving. In contrast, Balzano *et al*[4] reported that their patients with spleen preservation had less pancreatic leakage compared to patients with splenectomy (20% *vs* 38%, $P = 0.15$), while Lillemoe *et al*[23] reported that the patients who underwent a DP with splenectomy had a similar complication rate (30% *vs* 29%) compared to patients who underwent spleen preserving DP, but with no specific focus on pancreatic leak.

Finally, we did not find a relation between the onset of pancreatic fistula and demographic factors (age, gender) nor with the technique of pancreatic closure (there has not been any correlation between pancreatic texture and the dissection technique used) and the additional procedures. In our series, fistula occurred similarly in patients who underwent pancreatic resection only and in patients who underwent one or more additional procedures.

In conclusion, pancreatic fistula after DP affects 22% of patients. None of the two techniques we used seemed useful to significantly reduce this incidence. Based on these findings, we support that the role that technique (either stapler or suture) of pancreatic stump closure plays in the development of pancreatic leak is unclear. Both techniques are regarded as simple, quick and secure although the fistula rate remains high. However, there is a clearly determined relationship between the primary pathology, the octreotide therapy, the texture of the pancreatic parenchyma, the concomitant splenectomy and the postoperative pancreatic fistula formation.

REFERENCES

1 **Takeuchi K**, Tsuzuki Y, Ando T, Sekihara M, Hara T, Kori T, Nakajima H, Kuwano H. Distal pancreatectomy: is staple closure beneficial? *ANZ J Surg* 2003; **73**: 922-925

2 **Sheehan MK**, Beck K, Creech S, Pickleman J, Aranha GV. Distal pancreatectomy: does the method of closure influence fistula formation? *Am Surg* 2002; **68**: 264-267; discussion 267-268

3 **Sledzianowski JF**, Duffas JP, Muscari F, Suc B, Fourtanier F. Risk factors for mortality and intra-abdominal morbidity after distal pancreatectomy. *Surgery* 2005; **137**: 180-185

4 **Balzano G**, Zerbi A, Cristallo M, Di Carlo V. The unsolved problem of fistula after left pancreatectomy: the benefit of cautious drain management. *J Gastrointest Surg* 2005; **9**: 837-842

5 **Bassi C**, Dervenis C, Butturini G, Fingerhut A, Yeo C, Izbicki J, Neoptolemos J, Sarr M, Traverso W, Buchler M. Postoperative pancreatic fistula: an international study group (ISGPF) definition. *Surgery* 2005; **138**: 8-13

6 **Fernández-del Castillo C**, Rattner DW, Warshaw AL. Standards for pancreatic resection in the 1990s. *Arch Surg* 1995; **130**: 295-299; discussion 299-300

7 **Bilimoria MM**, Cormier JN, Mun Y, Lee JE, Evans DB, Pisters PW. Pancreatic leak after left pancreatectomy is reduced following main pancreatic duct ligation. *Br J Surg* 2003; **90**: 190-196

8 **Sugo H**, Mikami Y, Matsumoto F, Tsumura H, Watanabe Y, Futagawa S. Comparison of ultrasonically activated scalpel versus conventional division for the pancreas in distal pancreatectomy. *J Hepatobiliary Pancreat Surg* 2001; **8**: 349-352

9 **Fahy BN**, Frey CF, Ho HS, Beckett L, Bold RJ. Morbidity, mortality, and technical factors of distal pancreatectomy. *Am J Surg* 2002; **183**: 237-241

10 **Balcom JH**, Rattner DW, Warshaw AL, Chang Y, Fernandez-del Castillo C. Ten-year experience with 733 pancreatic resections: changing indications, older patients, and decreasing length of hospitalization. *Arch Surg* 2001; **136**: 391-398

11 **Shoup M**, Brennan MF, McWhite K, Leung DH, Klimstra D, Conlon KC. The value of splenic preservation with distal pancreatectomy. *Arch Surg* 2002; **137**: 164-168

12 **Suc B**, Msika S, Fingerhut A, Fourtanier G, Hay JM, Holmières F, Sastre B, Fagniez PL. Temporary fibrin glue occlusion of the main pancreatic duct in the prevention of intra-abdominal complications after pancreatic resection: prospective randomized trial. *Ann Surg* 2003; **237**: 57-65

13 **Kuroki T**, Tajima Y, Kanematsu T. Surgical management for the prevention of pancreatic fistula following distal pancreatectomy. *J Hepatobiliary Pancreat Surg* 2005; **12**: 283-285

14 **Konishi T**, Hiraishi M, Kubota K, Bandai Y, Makuuchi M, Idezuki Y. Segmental occlusion of the pancreatic duct with prolamine to prevent fistula formation after distal pancreatectomy. *Ann Surg* 1995; **221**: 165-170

15 **Kajiyama Y**, Tsurumaru M, Udagawa H, Tsutsumi K, Kinoshita Y, Akiyama H. Quick and simple distal pancreatectomy using the GIA stapler: report of 35 cases. *Br J Surg* 1996; **83**: 1711

16 **Bassi C**, Butturini G, Falconi M, Salvia R, Sartori N, Caldiron E. Prospective randomized pilot study of management of the pancreatic stump following distal pancreatectomy. *HPB* 1999; **1**: 203-207

17 **Degiannis E**, Levy RD, Potokar T, Lennox H, Rowse A, Saadia R. Distal pancreatectomy for gunshot injuries of the distal pancreas. *Br J Surg* 1995; **82**: 1240-1242

18 **Lowy AM**, Lee JE, Pisters PW, Davidson BS, Fenoglio CJ, Stanford P, Jinnah R, Evans DB. Prospective, randomized trial of octreotide to prevent pancreatic fistula after pancreaticoduodenectomy for malignant disease. *Ann Surg* 1997; **226**: 632-641

19 **Yeo CJ**, Cameron JL, Lillemoe KD, Sauter PK, Coleman J, Sohn TA, Campbell KA, Choti MA. Does prophylactic octreotide decrease the rates of pancreatic fistula and other complications after pancreaticoduodenectomy? Results of a prospective randomized placebo-controlled trial. *Ann Surg* 2000; **232**: 419-429

20 **Gouillat C**, Chipponi J, Baulieux J, Partensky C, Saric J, Gayet B. Randomized controlled multicentre trial of somatostatin infusion after pancreaticoduodenectomy. *Br J Surg* 2001; **88**: 1456-1462

21 **Büchler M**, Friess H, Klempa I, Hermanek P, Sulkowski U, Becker H, Schafmayer A, Baca I, Lorenz D, Meister R. Role of octreotide in the prevention of postoperative complications following pancreatic resection. *Am J Surg* 1992; **163**: 125-130; discussion 130-131

22 **Suc B**, Msika S, Piccinini M, Fourtanier G, Hay JM, Flamant Y, Fingerhut A, Fagniez PL, Chipponi J. Octreotide in the prevention of intra-abdominal complications following elective pancreatic resection: a prospective, multicenter randomized controlled trial. *Arch Surg* 2004; **139**: 288-294; discussion 295

23 **Lillemoe KD**, Kaushal S, Cameron JL, Sohn TA, Pitt HA, Yeo CJ. Distal pancreatectomy: indications and outcomes in 235 patients. *Ann Surg* 1999; **229**: 693-698; discussion 698-700

S- Editor Liu Y **L- Editor** Li M **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5101-5107
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Electrogastrography: Poor correlation with antro-duodenal manometry and doubtful clinical usefulness in adults

Shahab Abid, Greger Lindberg

Shahab Abid, Greger Lindberg, Karolinska Institutet, Department of Medicine, Division of Gastroenterology and Hepatology, Karolinska University Hospital Huddinge, Stockholm 14186, Sweden
Supported by funds from the Swedish Research Council (grant 2002-5489) and the Swedish Society of Medicine (Ihre's fond)
Correspondence to: Greger Lindberg, MD, PhD, Associate Professor, Karolinska Institutet, Department of Medicine, Division of Gastroenterology and Hepatology, Karolinska University Hospital, Huddinge, Stockholm 14186, Sweden. greger.lindberg@ki.se
Telephone: +46-85-8582316
Received: February 4, 2007 Revised: August 8, 2007

Abstract

AIM: To investigate if there is a correlation between electrical activity measured by electrogastrography (EGG) and contractile activity of the stomach as measured by antroduodenal manometry (ADM). We also studied whether the underlying motility disorder could be predicted from EGG parameters.

METHODS: We compared 21 parameters measured from EGG with 8 parameters measured from ADM. The ability of EGG to identify the underlying diagnosis was tested by comparing EGG parameters for each diagnosis group against other patients. The study comprised recordings from 148 patients and 125 females. Their median age was 45 (range 17-76) years.

RESULTS: We found few and weak correlations between EGG and ADM. Specifically the correlation between parameters reflecting the response to meal was poor (r = -0.07, P = 0.39). The discriminatory power of EGG for underlying motility disorder was also low. Patients with slow transit constipation (STC) showed a lower postprandial power in normogastric (3.7 ± 0.5 *vs* 4.0 ± 0.5) and tachygastric (3.5 ± 0.4 *vs* 3.7 ± 0.4) regions, a lower percentage of time with normogastria [87.2 (56.5-100)% *vs* 95.7 (0-100)%], and a higher percentage of time with tachygastria [9.3 (0-33)% *vs* 3.5 (0-100)%] and bradygastria [1.8 (0-20)% *vs* 0 (0-17.1)%]. Patients with irritable bowel syndrome had a higher percentage of time with normogastria [96.5 (62.5-100)% *vs* 93.3 (0-100)%] and a less unstable dominant frequency as measured by the instability coefficient [15 (3-77) *vs* 24 (2-72)].

CONCLUSION: EGG and ADM seem to measure different aspects of gastric motor activity but cannot show a spatial correlation. The diagnostic value of EGG is poor, but EGG may have some value for the identification of patients with STC.

Key words: Antroduodenal manometry; Correlation; Diagnostic use; Electrogastrography; Functional bowel disorders; Physiopathology

Abid S, Lindberg G. Electrogastrography: Poor correlation with antro-duodenal manometry and doubtful clinical usefulness in adults. *World J Gastroenterol* 2007; 13(38): 5101-5107

http://www.wjgnet.com/1007-9327/13/5101.asp

INTRODUCTION

Electrogastrography (EGG) is a technique for recording gastric myoelectric activity using cutaneous electrodes placed on the abdominal wall overlying the stomach. EGG detects rhythm and power (amplitude) of gastric myoelectricity[1]. Studies have shown a good correlation between cutaneous EGG recordings and myoelectric signals recorded from gastric serosal leads[2]. However, there are major concerns regarding the clinical usefulness of this non-invasive procedure[3].

EGG has been advocated as a diagnostic test in the clinical evaluation of patients with unexplained nausea, vomiting and other dyspeptic symptoms to gain insight into the mechanisms of symptom generation. Conflicting results have been obtained in previous studies. In some studies, subsets of patients with upper abdominal symptoms have depicted prominent EGG abnormalities whereas healthy volunteers rarely have exhibited EGG rhythm or power disturbances[4,5]. Other studies have found no difference in EGG parameters between dyspeptic patients and healthy volunteers[6,7].

Patients with systemic diseases such as Parkinson's disease[8], myotonic dystrophy[9], and diabetes mellitus[10] show abnormal EGG findings. However, in patients with progressive systemic sclerosis (which is usually associated with poor gut motility), EGG shows unchanged parameters[11]. Similarly, EGG shows a greater variability compared to normal volunteers but no typical EGG pattern could be identified for different surgical procedures in surgical patients after cholecystectomy, Nissen fundoplication, subtotal gastrectomy or vagotomy and gastric pull-up operations[12].

Antroduodenal manometry (ADM) records lumen-occluding contractions of the gastric antrum[13]. Under physiological conditions there is a temporal correlation between myoelectric slow waves and contractile activity of the antrum. It is less clear if there is a spatial correlation between electrical and contractile activity. A small study in healthy females showed an unstable EGG peak power during fasting and an increase in EGG peak power after a solid test meal as indicative of a correlation between electrical activity and gastric motor activity[14]. It is conceivable that there should be a correlation between electrical activity measured by EGG and contractile activity measured by ADM. It would be of interest to know if EGG can predict gastric contractile activity. Surprisingly, a direct head to head comparison between EGG and ADM has not been done previously.

The aim of the present study was to compare measures of electrical activity determined by EGG and antral contractile activity from antroduodenal manometry. We also investigated whether EGG could differentiate between different underlying diagnoses of motility disorders.

MATERIALS AND METHODS

Subjects

This was a retrospective analysis of data collected during 1994-2001. Adult patients suffering from various functional and motility disorders of the gastrointestinal tract were included in our study. All of them were subjected to a combined EGG and ADM study as part of their clinical work-up for suspected gastrointestinal motility disorders. The two measurements were done simultaneously using two different systems, thus synchronous analysis was not possible in this study.

Electrogastrography

This procedure has been described elsewhere[6]. Briefly, a single bipolar channel was used. The EGG signal was recorded using a specially designed digital recorder (Digitrapper-EGG, Medtronic Synectics, Stockholm, Sweden) and sampled at a rate of 1 Hz. EGG recordings were examined visually for artefacts and then categorized into excellent (no artefacts), good (< 25% of recording time excluded because of artefacts), fair (< 50% excluded) or poor quality (≥ 50% excluded). After exclusion of artefacts, the remaining parts of the recordings were analysed with a software provided by the vendor (Polygram version 6.40, Medtronic Synectics, Stockholm, Sweden). This program could analyse the EGG using fast Fourier transformations of 256-s runs with an overlap of 196 s, so-called running spectral analysis, and can also make a Fourier transformation of an entire period in order to obtain a single power spectrum for that period. The parameters derived from the automated analysis of EGG are shown in Table 1. Dominant frequency instability coefficient (DFIC) and dominant power instability coefficient (DPIC) are measures of the variability of the EGG signal, which were computed as the standard deviations divided by the mean value of the dominant frequency and dominant power, respectively, from the running spectral analysis of each period.

Table 1 Electrogastrography (EGG) and antroduodenal manometry (ADM) variables

1	EGG variables
	(1) Preprandial (fasting) and postprandial percentage of time with dominant frequency in:
	Bradygastria (0.5-2 cycles per minute)
	Normogastria (2-4 cycles per minute) (0.5-2 cycles per minute)
	Tachygastria (4-10 cycles per minute)
	(2) Pre- and postprandial power attributed to the three frequency bands (bradygastria, tachygastria and normogastria)
	(3) Pre- and postprandial dominant power instability co-efficient (DPIC)
	(4) Pre- and postprandial dominant frequency instability co-efficient (DFIC)
	(5) Pre- and postprandial dominant frequency (DF)
	(6) Pre and postprandial period dominant power (PDP)
	(7) Power ratio (ratio of postprandial to preprandial power of dominant frequency)
2	ADM variables
	(1) Pre- and postprandial motility index (MI)
	(2) Pre- and postprandial contractile frequency
	(3) Pre- and postprandial median amplitude
	(4) MI ratio (ratio between postprandial and preprandial motility index)
	(5) Amplitude ratio (ratio between postprandial and preprandial amplitude)

Antroduodenal manometry

ADM was performed using a flexible catheter with either 6 or 8 water-perfused channels. The 6-channel catheter has 3 channels spaced 2 cm apart for the recording of antral motor activity whereas the 8-channel catheter has 5 channels spaced 1 cm apart for the same purpose. We used a pneumo-hydraulic pump (Arndorfer Medical Specialties, Greendale, WI, USA) with catheters connected to external pressure transducers. Data logging was done with a Polygraph 12HR and the Polygram (Medtronic Synectics, Stockholm, Sweden). The protocol included a 3-hour fasting period followed by a test meal (500 kcal standardized mixed meal) and 2-hour post-prandial recording[15]. Numerical analysis was done on data from the most distal recording site in the gastric antrum. Data were extracted using the Polygram that was set to detect contractions with an amplitude > 9 mmHg. We determined the mean frequency and amplitude of contractions and motility index during the last hour before and the first hour after intake of the test meal. The motility index was calculated as the area under the curve above baseline per time unit. We compared 8 variables from ADM with 21 variables from EGG (Table 1).

Statistical analysis

Normally distributed and lognormal data were expressed as mean ± SD and data with a non-normal distribution were given as a median and full range. We used Student's *t*-test and Mann-Whitney *U*-test for assessing differences between the groups. Correlations were studied using Pearson's product moment correlation. Logistic regression analysis was done to test EGG parameters for their discriminatory value in predicting underlying diagnoses. $P < 0.05$ was considered statistically significant.

Table 2 Correlation matrix (*r*-values) for parameters from antroduodenal manometery and electrogastrography (*n* = 144)

Parameters	Pre MI[1]	Post MI[1]	MI ratio[§]	Pre Freq	Post Freq	Pre Amp[1]	Post Amp[1]	Amp Ratio[1]
Pre-Brady power[1]	0.15	0.01	-0.10	0.10	0.01	0.08	0.06	-0.01
Pre-Normo power[1]	0.06	0.03	-0.01	0.10	0.05	-0.01	-0.01	0.00
Pre-Tachy power1	0.10	0.05	-0.03	0.13	0.07	0.01	0.00	-0.01
Pre-Brady DF%	0.06	-0.01	-0.05	0.05	0.01	-0.09	0.08	0.12
Pre-Normo DF%	0.05	0.09	0.06	0.08	0.08	0.06	0.14	0.06
Pre-Tachy DF%	-0.03	-0.13	-0.11	-0.07	-0.12	-0.02	-0.18[a]	-0.11
Pre-DFIC	-0.04	0.04	0.06	-0.12	0.02	0.07	0.03	-0.04
Pre-DPIC	-0.01	-0.13	-0.12	-0.12	-0.13	0.09	-0.10	-0.14
Pre-DF	-0.08	-0.11	-0.05	-0.10	-0.10	-0.04	-0.17[a]	-0.09
Pre-PDP[1]	-0.09	0.01	0.08	0.00	0.03	-0.02	-0.04	-0.02
Post-Brady power[1]	0.04	-0.04	-0.07	0.13	-0.04	-0.10	0.02	0.09
Post-Normo power[1]	0.09	-0.01	-0.07	0.13	0.04	-0.03	-0.04	-0.01
Post-Tachy power[1]	0.08	0.01	-0.05	0.15	0.03	-0.06	-0.02	0.03
Post-Brady DF%	0.00	0.01	0.01	-0.02	-0.07	-0.06	0.01	0.05
Post-Normo DF%	-0.03	-0.03	-0.01	-0.06	-0.04	0.06	0.06	0.00
Post-Tachy DF%	0.02	-0.04	-0.05	0.05	-0.02	-0.05	-0.09	-0.02
Post-DFIC	0.04	0.03	-0.01	0.11	0.08	-0.09	-0.11	-0.01
Post-DPIC	-0.06	-0.1[a]	-0.14	-0.09	-0.17[a]	0.02	-0.18[a]	-0.15
Post-DF	0.04	-0.01	-0.04	0.06	0.00	-0.05	0.00	0.04
Post-PDP[1]	0.06	-0.01	-0.04	0.08	0.04	0.01	-0.02	-0.03
Power ratio[1]	0.04	-0.04	-0.07	0.04	-0.01	-0.02	-0.04	-0.01

[1]Log values, [a]$P < 0.05$.

RESULTS

A total of 185 patients were eligible for the study but 37 were excluded either because of previous gastric resection ($n = 6$), poor quality of the EGG recording ($n = 23$), or because of a technical failure ($n = 8$) in the EGG system. Another 4 patients were excluded from the comparison between EEG and ADM because ADM recordings did not show a recording from the antrum during the fasting or the fed period or both. Thus, the study population finally included 148 patients for comparing EGG parameters among various diagnosis groups and 144 patients for assessing the correlation between EGG and ADM parameters. The median age of the patients was 45 (range 17-76) years and 122 (82%) were females. There were 52 patients (35%) with IBS, 22 (15%) with enteric dysmotility (ED), i.e. abnormal small bowel motor activity but no bowel dilatation[16], 26 (18%) with slow transit constipation (STC), 11 (7%) with chronic intestinal pseudo-obstruction (CIP), 13 (9%) with functional dyspepsia with or without gastroparesis and 24 (16%) with other diagnoses, including various systemic and neurological diseases.

Comparison of electrogastrography and antral manometry

Few correlations were found between EGG and ADM. The correlation matrix was analyzed between 21 EGG parameters and 8 ADM parameters in this study (Brady = bradygastria, Normo = normogastria, Tachy = tachygastria, DF = dominant frequency, DFIC = dominant frequency instability coefficient, DPIC = dominant power instability coefficient, PDP = period dominant power, Amp = amplitude, MI = motility index, Freq = frequency, Pre = preprandial, Post = postprandial) (Table 2).

Postprandial contractile activity (amplitude, frequency and motility index) showed weak, negative correlations with the postprandial dominant power instability coefficient. Preprandial tachygastria (reflected by the percentage of time with a dominant frequency in the tachygastric region and dominant frequency) was also negatively correlated with postprandial contractile amplitude. The comparison between EGG response to meal ingestion (ratio between postprandial and fasting power) and ADM response to ingestion of a 500 kcal test meal (ratio between postprandial and fasting motility index) revealed no correlation ($r = -0.07$, $P = 0.44$, Figure 1).

Electrogastrography and underlying diagnosis

The ability of EGG to identify diagnostic groups was tested by comparing EGG parameters for each group against all other patients included. Table 3 shows the discriminatory power of EGG for the larger patient groups. Variables with a normal or log-normal distribution were expressed as mean ± SD, else as median and full range. Patients with other diagnoses ($n = 24$) were not listed but included in the calculation of differences. Significant *P*-values were expressed as [a]$P < 0.05$, [b]$P < 0.01$, and [d]$P < 0.001$ (Brady: bradygastria, Tachy: tachygastria, Normo = normogastria, DFIC: dominant frequency instability coefficient, DPIC: dominant power instability coefficient, DF: dominant frequency, PDP: period dominant power).

In general, the discriminatory power of EGG was low. Only patients with STC in one diagnostic group, showed a reasonable number of differences in postprandial EGG parameters compared to those in all other diagnosis groups. STC patients had a lower postprandial power in the normogastric region (3.7 ± 0.5 *vs* 4.0 ± 0.5, $P < 0.01$) and in the tachygastric region (3.5 ± 0.4 *vs* 3.7 ± 0.4, $P < 0.05$). Similarly, the postprandial percentage of time with a dominant frequency was higher in bradygastric and tachygastric frequency bands and lower in the normogastric frequency band of STC patients (Table 3). Patients with STC also demonstrated a significantly lower postprandial dominant power (2.1 ± 0.6 *vs* 2.4 ± 0.5) compared to other patients.

In patients with CIP, EGG during fasting showed a sig-

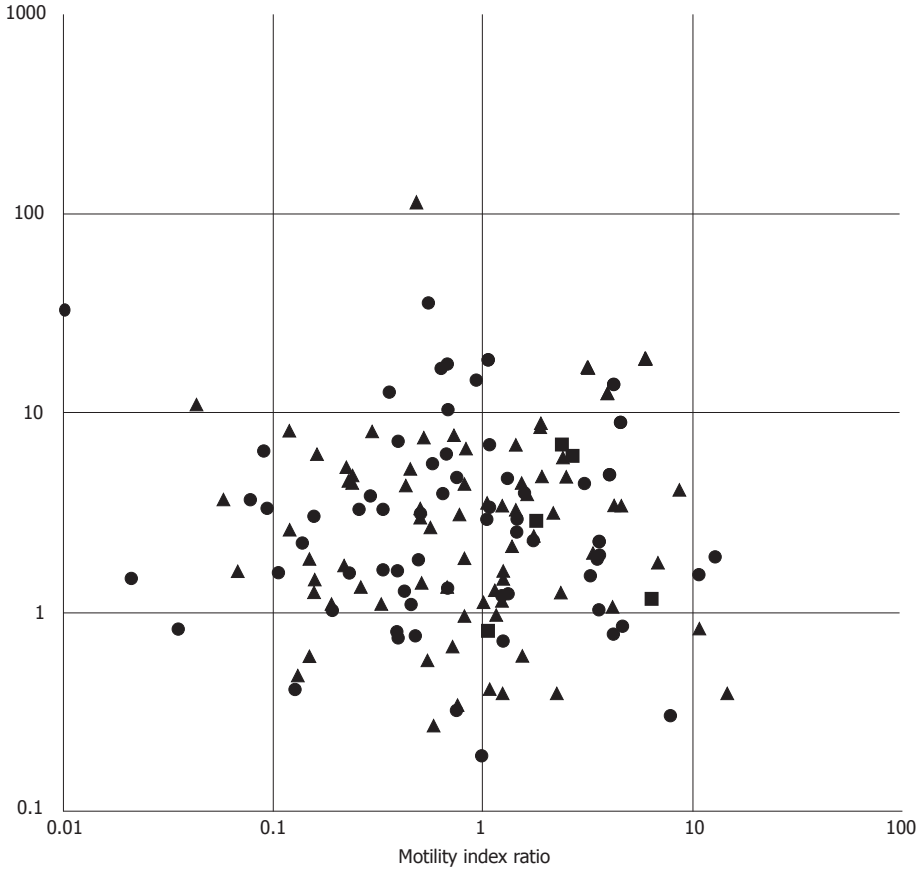


Figure 1 Scatterplot of motility index ratio and power ratio in 144 patients (logarithmic scales). Symbols indicate different qualities of the EGG record: ■ = excellent; ● = good; and ▲ = fair quality.

Table 3 Electrogastrography parameters among various diagnostic groups

EGG parameter	Chronic intestinal pseudo-obstruction (n = 11)	Enteric dysmotility (n = 22)	Irritable bowel syndrome (n = 52)	Functional dyspepsia/ gastroparesis (n = 13)	Slow transit constipation (n = 26)
Preprandial					
Log Brady power	2.9 ± 0.4	2.8 ± 0.5	2.7 ± 0.5	2.7 ± 0.4	2.6 ± 0.4
Log Normo power	3.8 ± 0.5[a]	3.7 ± 0.5	3.6 ± 0.5	3.6 ± 0.5	3.5 ± 0.5
Log Tachy power	3.7 ± 0.4[a]	3.5 ± 0.5	3.3 ± 0.4	3.4 ± 0.4	3.3 ± 0.4
Brady DF%	2.7 (0-16.2)	1.8 (0-17.5)	0 (0-15.2)	0 (0-10.3)	2 (0-14.3)
Normo DF%	89.7 (0-100)	88.2 (36.2-100)	92.0 (38.6-100)	89.8 (59-100)	87.7 (30.8-100)
Tachy DF%	6.0 (0-100)	8.8 (0-61.7)	5.1 (0-61.4)	6.5 (0-73.7)	6.1 (0-69.2)
DFIC	22 (3-51)	32 (5-68)	23 (3-75)[a]	31 (1-70)	30 (3-69)
DPIC	77 (40-165)	67 (33-169)	64 (39-145)	65 (31-101)	60 (47-159)
DF	3.05 (2.58-11.25)	3.05 (2.34-3.98)	3.05 (2.11-6.05)	3.05 (2.58-3.75)	3.05 (2.11-8.91)
Log PDP	2.4 ± 0.6[a]	2.0 ± 0.5	2.1 ± 0.6	2.1 ± 0.5	1.9 ± 0.5
Postprandial					
Log Brady power	3.0 ± 0.4	3.0 ± 0.5	2.9 ± 0.4	2.9 ± 0.5	2.8±0.5
Log Normo power	4.1 ± 0.3	4.1 ± 0.5	4.0 ± 0.5	4.1 ± 0.7	3.7 ± 0.5[b]
Log Tachy power	3.9 ± 0.4	3.8 ± 0.5[a]	3.6 ± 0.4	3.8 ± 0.6	3.5 ± 0.4[a]
Brady DF%	0 (0-5.8)	0 (0-5.0)	0 (0-14.5)	0 (0-17.1)	1.8 (0-20)[b]
Normo DF%	92.3 (0-100)	96.1 (32.1-100)	96.5 (62.5-100)[a]	95.5 (32-100)	87.2 (56.5-100)[d]
Tachy DF%	5.8 (0-100)	1.8 (0-66)	3.1 (0-18.8)	1.9 (0-21.1)	9.3 (0-33)[b]
DFIC	18 (4-63)	16 (2-62)	15 (3-77)[a]	27 (5-56)	27 (8-72)[b]
DPIC	67 (59-81)	56 (35-110)	70 (34-129)	61 (30-82)	64 (39-108)
DF	3.05 (2.58-9.14)	3.28 (2.34-9.61)	3.05 (2.58-3.75)	3.28 (2.11-10.08)	3.05 (2.34-3.75)
Log PDP	2.5 ± 0.5	2.5 ± 0.5	2.4 ± 0.5	2.5 ± 0.8	2.1 ± 0.6[b]
Log Power ratio	0.2 ± 0.5	0.5 ± 0.4	0.4 ± 0.4	0.5 ± 0.6	0.2 ± 0.5

nificantly higher power in the tachygastric region (3.7 ± 0.4 *vs* 3.3 ± 0.4, $P < 0.05$) and a higher period dominant power (2.4 ± 0.7 *vs* 2.0 ± 0.5) compared to patients without CIP. In patients with IBS there was a higher percentage of time with a normogastric dominant frequency in the postprandial period and a more stable dominant frequency measured by DFIC during both preprandial and postprandial periods (Table 3).

Twenty patients underwent gastric emptying studies. Five of them had delayed gastric emptying. No significant differences were found in EGG parameters among patients with or without delayed gastric emptying.

DISCUSSION

To the best of our knowledge, this is the largest series of simultaneous recordings of EGG and ADM in patients with motility disorders of the gut. In the present study, we studied the relationship between EGG parameters and antral manometric findings. We also tried to evaluate the role of EGG in discriminating between various motility disorders of the gastrointestinal tract. EGG is not capable to record spike activity, hence the major electrical indicator of contractile activity is absent in its recordings. However, an increase in wave amplitude on EGG (referred to as power from the spectral analysis) can be asserted as a reflection of contractile activity. It has been observed that EGG power increases after certain drugs or meals that stimulate motility and decreases after drugs or meals that inhibit motility[12]. Therefore, to measure the significance of EGG power, it is best to compare EGG changes before and after the test meal given during EGG recording[14]. We found few and weak correlations between ADM findings and EGG parameters, especially no correlation between the motility index ratio and the power ratio. This is contrary to the findings of another study[17], where the EGG power ratio was significantly but weakly correlated with the motility index ratio. The number of patients in that study was, however, only 16. A partial correlation has also been found in children between the postprandial increase in amplitude of gastric electrical activity and antral motor activity[18]. In the present study, a more stable dominant power, i.e. lower DPIC, was associated with a higher contractile activity and tachygastria during the preprandial period was associated with lower amplitudes of contraction during the postprandial period, but the correlations were weak in both cases.

One important reason for a poor correlation between EGG-recorded electrical activity and ADM-recorded motor activity of the stomach could be that EGG recordings are sensitive to the degree of gastric wall distension and its approximation to the anterior abdominal wall. An underlying motility disorder of the gut might cause the gastric wall to distend inappropriately in response to the test meal and therefore lead to an inadequate EGG recording of gastric myoelectric activity. In light of the weak correlation between ADM and EGG found in other studies[17,18] and our observations, we think that EGG and ADM can measure two different aspects of gastric motor activity and a spatial relation cannot be fully determined between EGG and ADM.

Surprisingly, STC in the only diagnostic group stood out as clearly different from the other diagnostic groups on the basis of EGG parameters. Patients with STC exhibited differences mainly in the postprandial EGG parameters. Many studies have demonstrated a decrease in the density of interstitial cells of Cajal (ICC) in patients with slow transit constipation[19-21]. It is possible that observed postprandial changes in EGG parameters might be the reflection of a more generalized gastrointestinal myoelectrical dysfunction in STC patients secondary to depletion of ICC and other neuroenteric abnormalities. Further studies are needed to validate the EGG findings in patients with STC in the present study.

It was reported that EGG is diagnostically useful in adult patients with CIP[22]. Moreover, a comparison between normal subjects and children with CIP could reveal a significant increase of preprandial values for tachygastria and period dominant frequency and a decrease in normogastria and an increase in total tachygastria values in postprandial period[23]. In our study, the CIP patients had a significantly higher power on EGG in the tachygastric region and a higher PDP during fasting.

Another field of interest among researchers interested in evaluating the clinical usefulness of EGG is functional dyspepsia with or without gastroparesis. Studies have shown abnormal EGG patterns such as abnormal slow wave propagation and coupling[24], lower power ratio, lower postprandial level of increment in dominant power and a higher instability coefficient[25,26] in patients with functional dyspepsia. However, abnormal parameters are not the same in different studies. Interestingly, significantly more gastric arrhythmias have been found in oesophageal reflux patients with dyspeptic symptoms than in those with no dyspeptic symptoms[27].

Contradictory findings can ben found when capability of EGG is assessed to predict delayed gastric emptying. It was reported that patients with impaired gastric emptying show a significantly lower percentage of normal gastric slow waves and a lower postprandial increment in dominant power[28]. On the other hand, another study conducted in patients with systemic sclerosis has not found any correlation between delayed gastric emptying and EGG abnormalities[11]. In our study, no difference in EGG parameters was found between dyspeptic patients with delayed gastric emptying and those with normal gastric emptying, possibly due to a small number of patients with gastric emptying.

Our study has certain limitations. One of them was the retrospective design of the present series, which is unlikely to have influenced the results of the present study concerning the correlation between EGG and ADM. On the other hand, the comparison of EGG parameters between different diagnostic groups was hampered by a small number of patients in certain groups. Another limitation was the absence of a control group to compare with patients with motility disorders. In the present series, however, comparison of both EGG and ADM comparison was available in each patient. In our opinion, the assessment of correlation between EGG and ADM is not affected by the absence of a control group. We used a single channel EGG instead of a multiple channel EGG in our study. Novel multi-channel EGG can study the propagation and coupling of slow waves in the stomach, and is perhaps more helpful than single channel EGG in the diagnosis of gastric dysfunction[24]. It is possible that registrations from multiple channels would have a better chance to detect correlations with contractile activity, not least because sampling error from multiple channels would be smaller than those from a single channel. However, no data are available from comparisons between ADM and multi-channel EGG.

A high exclusion rate of patients in our series was due to EGG recordings that were not interpretable. Motion artefacts were common despite the stationary condition of the patients. Care was taken in preparing the skin so that the impedance between any two electrodes was less than

10 ohms. Nevertheless, many recordings exhibited low frequency aberrations and shifts of baseline. Moreover, various technical problems, such as faulty electrode cables or malfunction due to static electricity, also resulted in exclusion of a significant number of patients from analysis. We have not come across any account for such failures of EGG recording in previously published studies.

In short, no consistent relationship was observed between EGG and ADM in this series. No evidence was found in this study to favour a spatial correlation between the parameters measured by EGG and ADM. Moreover, the ability of EGG to identify motility disorders was generally poor in this study.

In conclusion, EGG findings in STC patients are interesting but further studies are required to validate the role of EGG in STC patients.

COMMENTS

Background

Electrogastrography is to measure the electrical activity of the stomach from electrodes attached to the abdominal surface. The usefulness of this technique for the diagnosis of gastrointestinal diseases is yet unknown. However, since electrical activity is pivotal for motor function, it is believed that measurement of electrical activity should reveal important diagnostic information in patients with motility disorders.

Research frontiers

The correlation between measures derived from electrogastrography and other techniques such as manometry has received little attention. Such correlations are important for understanding the relation between different measurement techniques.

Innovations and breakthroughs

This is a head-to-head comparison of electrogastrography and antroduodenal manometry, i.e. measurement of contractile activity of the stomach done at the same time from a large number of patients with gastrointestinal motility disorders.

Applications

This study could not show any correlation between antroduodenal manometry and electrogastrography, suggesting that the two techniques can measure different aspects on gastric motor function. The study also showed that the ability of electrogastrography to identify patients with different diagnoses was poor. To the surprise of the authors, patients with slow transit constipation, a severe form of intractable constipation, were the only group of patients identified by electrogastrography. The reason for this is not clear, but it is known from previous research that patients with slow transit constipation have a lack of pacemaker cells in the gut, which could explain why they also had disturbed electrical rhythm in the stomach.

Terminology

Correlation is a measure of the relative correspondence of two sets of data. Manometry is the measurement of (in this case, intraluminal) pressure over time. Motility disorders comprise a large number of disorders that affect gastrointestinal motor activity. The most common disorder is irritable bowel syndrome.

Peer review

This is a well written paper on a subject with a considerable debate. Whether electrogastrography and antroduodenal manometry can measure different aspects of gastric motor activity needs to be further studied.

REFERENCES

1 **Chang FY**. Electrogastrography: basic knowledge, recording, processing and its clinical applications. *J Gastroenterol Hepatol* 2005; **20**: 502-516

2 **Lin Z**, Chen JD, Schirmer BD, McCallum RW. Postprandial response of gastric slow waves: correlation of serosal recordings with the electrogastrogram. *Dig Dis Sci* 2000; **45**: 645-651

3 **Bortolotti M**. Electrogastrography: a seductive promise, only partially kept. *Am J Gastroenterol* 1998; **93**: 1791-1794

4 **Ogawa A**, Mizuta I, Fukunaga T, Takeuchi N, Honaga E, Sugita Y, Mikami A, Inoue Y, Takeda M. Electrogastrography abnormality in eating disorders. *Psychiatry Clin Neurosci* 2004; **58**: 300-310

5 **Rezende Filho J**, De Rezende JM, Melo JR. Electrogastrography in patients with Chagas' disease. *Dig Dis Sci* 2005; **50**: 1882-1888

6 **Holmvall P**, Lindberg G. Electrogastrography before and after a high-caloric, liquid test meal in healthy volunteers and patients with severe functional dyspepsia. *Scand J Gastroenterol* 2002; **37**: 1144-1148

7 **Oba-Kuniyoshi AS**, Oliveira Jr JA, Moraes ER, Troncon LE. Postprandial symptoms in dysmotility-like functional dyspepsia are not related to disturbances of gastric myoelectrical activity. *Braz J Med Biol Res* 2004; **37**: 47-53

8 **Lin Z**, Eaker EY, Sarosiek I, McCallum RW. Gastric myoelectrical activity and gastric emptying in patients with functional dyspepsia. *Am J Gastroenterol* 1999; **94**: 2384-2389

9 **Rönnblom A**, Hellström PM, Holst JJ, Theodorsson E, Danielsson A. Gastric myoelectrical activity and gut hormone secretion in myotonic dystrophy. *Eur J Gastroenterol Hepatol* 2001; **13**: 825-831

10 **Koch KL**. Electrogastrography: physiological basis and clinical application in diabetic gastropathy. *Diabetes Technol Ther* 2001; **3**: 51-62

11 **Franck-Larsson K**, Hedenström H, Dahl R, Rönnblom A. Delayed gastric emptying in patients with diffuse versus limited systemic sclerosis, unrelated to gastrointestinal symptoms and myoelectric gastric activity. *Scand J Rheumatol* 2003; **32**: 348-355

12 **Kauer WK**, Stein HJ, Balint A, Siewert JR. Transcutaneous electrogastrography: a non-invasive method to evaluate post-operative gastric disorders? *Hepatogastroenterology* 1999; **46**: 1244-1248

13 **Soffer EE**, Thongsawat S, Ellerbroek S. Prolonged ambulatory duodeno-jejunal manometry in humans: normal values and gender effect. *Am J Gastroenterol* 1998; **93**: 1318-1323

14 **Chen JD**, Richards RD, McCallum RW. Identification of gastric contractions from the cutaneous electrogastrogram. *Am J Gastroenterol* 1994; **89**: 79-85

15 **Stanghellini V**, Camilleri M, Malagelada JR. Chronic idiopathic intestinal pseudo-obstruction: clinical and intestinal manometric findings. *Gut* 1987; **28**: 5-12

16 **Wingate D**, Hongo M, Kellow J, Lindberg G, Smout A. Disorders of gastrointestinal motility: towards a new classification. *J Gastroenterol Hepatol* 2002; **17** Suppl: S1-S14

17 **Lee JS**, Jang JY, Hong SJ, Im HH, Ryu CB, Kim JO, Cho JY, Lee MS, Shim CS, Kim BS. Clinical significance of cutaneous electrogastrography (EGG) compared with antroduodenal manometry (ADM). *Neurogastroenterol Motil* 2002; **14**: 308

18 Faure C, Wolff VP, Navarro J. Effect of meal and intravenous erythromycin on manometric and electrogastrographic measurements of gastric motor and electrical activity. *Dig Dis Sci* 2000; **45**: 525-528

19 **Lee JI**, Park H, Kamm MA, Talbot IC. Decreased density of interstitial cells of Cajal and neuronal cells in patients with slow-transit constipation and acquired megacolon. *J Gastroenterol Hepatol* 2005; **20**: 1292-1298

20 **Bassotti G**, Villanacci V, Maurer CA, Fisogni S, Di Fabio F, Cadei M, Morelli A, Panagiotis T, Cathomas G, Salerni B. The role of glial cells and apoptosis of enteric neurones in the neuropathology of intractable slow transit constipation. *Gut* 2006; **55**: 41-46

21 **Tong WD**, Liu BH, Zhang LY, Xiong RP, Liu P, Zhang SB. Expression of c-kit messenger ribonucleic acid and c-kit protein in sigmoid colon of patients with slow transit constipation. *Int J Colorectal Dis* 2005; **20**: 363-367

22 **Debinski HS**, Ahmed S, Milla PJ, Kamm MA. Electrogastrography in chronic intestinal pseudoobstruction. *Dig Dis Sci* 1996; **41**: 1292-1297
23 **Bracci F**, Iacobelli BD, Papadatou B, Ferretti F, Lucchetti MC, Cianchi D, Francalanci P, Ponticelli A. Role of electrogastrography in detecting motility disorders in children affected by chronic intestinal pseudo-obstruction and Crohn's disease. *Eur J Pediatr Surg* 2003; **13**: 31-34
24 **Lin X**, Chen JZ. Abnormal gastric slow waves in patients with functional dyspepsia assessed by multichannel electrogastrography. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G1370-G1375
25 **Lu CL**, Chen CY, Chang FY, Kang LJ, Lee SD, Wu HC, Kuo TS. Impaired postprandial gastric myoelectrical activity in Chinese patients with nonulcer dyspepsia. *Dig Dis Sci* 2001; **46**: 242-249
26 **van der Voort IR**, Osmanoglou E, Seybold M, Heymann-Mönnikes I, Tebbe J, Wiedenmann B, Klapp BF, Mönnikes H. Electrogastrography as a diagnostic tool for delayed gastric emptying in functional dyspepsia and irritable bowel syndrome. *Neurogastroenterol Motil* 2003; **15**: 467-473
27 **Chen CL**, Lin HH, Huang LC, Huang SC, Liu TT. Electrogastrography differentiates reflux disease with or without dyspeptic symptoms. *Dig Dis Sci* 2004; **49**: 715-719
28 **Chen JD**, Lin Z, Pan J, McCallum RW. Abnormal gastric myoelectrical activity and delayed gastric emptying in patients with symptoms suggestive of gastroparesis. *Dig Dis Sci* 1996; **41**: 1538-1545

S- Editor Liu Y **L- Editor** Wang XL **E- Editor** Li JL

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5108-5115
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

24-hour esophageal pH-monitoring in children suspected of gastroesophageal reflux disease: Analysis of intraesophageal pH monitoring values recorded in distal and proximal channel at diagnosis

Janusz Semeniuk, Maciej Kaczmarski

Janusz Semeniuk, Maciej Kaczmarski, III Department of Pediatrics, Medical University of Białystok, Poland
Supported by Grant of State Committee for Scientific Research (KBN) No 4P05E 04719
Correspondence to: Janusz Semeniuk, MD, PhD, III Department of Pediatrics, Medical University of Białystok, Waszyngtona 17 street, 15-274 Białystok, Poland. Janexik@poczta.onet.pl
Telephone: +48-85-7423841 Fax: +48-85-7423841
Received: June 14, 2007 Revised: July 28, 2007

Abstract

AIM: To assess values of 24-h esophageal pH-monitoring parameters with dual-channel probe (distal and proximal channel) in children suspected of gastroesophageal reflux disease (GERD).

METHODS: 264 children suspected of gastroesophageal reflux (GER) were enrolled in a study (mean age χ = 20.78 ± 17.23 mo). The outcomes of this study, immunoallerrgological tests and positive result of oral food challenge test with a potentially noxious nutrient, enabled to qualify children into particular study groups.

RESULTS: 32 (12.1%) infants (group 1) had physiological GER diagnosed. Pathological acid GER was confirmed in 138 (52.3%) children. Primary GER was diagnosed in 76 (28.8%) children (group 2) and GER secondary to allergy to cow milk protein and/or other food (CMA/FA) in 62 (23.5%) children (group 3). 32 (12.1%) of them had CMA/FA (group 4-reference group), and in remaining 62 (23.5%) children neither GER nor CMA/FA was confirmed (group 5). Mean values of pH monitoring parameters measured in distal and proximal channel were analyzed in individual groups. This analysis showed statistically significant differentiation of mean values in the case of: number of episodes of acid GER, episodes of acid GER lasting > 5 min, duration of the longest episode of acid GER in both channels, acid GER index total and supine in proximal channel. Statistically significant differences of mean values among examined groups, especially between group 2 and 3 in the case of total acid GER index (only distal channel) were confirmed.

CONCLUSION: 24-h esophageal pH monitoring confirmed pathological acid GER in 52.3% of children with typical and atypical symptoms of GERD. The similar pH-monitoring values obtained in group 2 and 3 confirm the necessity of implementation of differential diagnosis for primary *vs* secondary cause of GER.

Key words: Children; Gastroesophageal reflux disease; 24-h esophageal pH-monitoring; 2-channel probe; Gastroesophageal reflux; Primary and secondary; CMA/FA; Oral food challenge test

Semeniuk J, Kaczmarski M. 24-hour esophageal pH-monitoring in children suspected of gastroesophageal reflux disease: Analysis of intraesophageal pH monitoring values recorded in distal and proximal channel at diagnosis. *World J Gastroenterol* 2007; 13(38): 5108-5115

http://www.wjgnet.com/1007-9327/13/5108.asp

INTRODUCTION

Acid gastroesophageal reflux (GER) in children at any age, could be the reason of various clinical manifestation (typical and atypical) of variable intensity, dependent on the range of reflux (high reflux, low reflux)[1-9]. On the basis of reflux symptoms it is hardly possible to differentiate primary GER from GER secondary to allergy to cow milk protein and/or other food (CMA/FA)[10-13]. In children suspected of gastroesophageal reflux disease (GERD), 24-h esophageal pH-monitoring is a diagnostic procedure that enables to confirm or exclude pathological reflux of acid gastric contents into esophagus i.e. acid GER[14-20]. Diagnostic procedure, including food allergy contribution in GER, is necessary to distinguish primary GER from secondary GER. Such procedure includes immunoallergological tests i.e. Prick tests, cIgE, sIgE[2,12,21-25]. Positive result of food oral challenge test with potentially noxious nutrient (biological trial) confirmed contribution of food allergy in triggering off reflux symptoms[25,26]. Comparative analysis of 24-h intraesophageal pH monitoring with dual-channel probe (distal and proximal channel) in children suspected of gastroesophageal reflux disease (GERD).

MATERIALS AND METHODS

264 children suspected of GERD, of both sexes (140 boys-53.0% and 124 girls-47.0%) were enrolled in a study. Patients were 1.5 mo to 102 mo old, mean age χ = 20.78 ± 17.23 mo. Information gathered at interview revealed that various gastrointestinal diseases appeared in family histories of all patients.

24-h esophageal pH monitoring

24-h intraesophageal pH-monitoring was performed with antimony dual-channel pH monitoring probe (distal channel/distal and proximal channel) and a device recording the values Digitraper MK Ⅲ, Synectics Medical. Antimony electrode was calibrated with buffer solutions of pH = 7.0 and pH = 1.0. pH-monitoring probe, 2.1 diameter, was positioned in esophagus through one of the nostrils and pharynx with distal channel (2) at the height of 3-5 cm, and proximal channel (1) at 10, 15 or 20 cm above the upper edge of lower esophageal sphincter (LES). To localize the probe Strobel's mathematical mode, radiological examination, and manometry of LES were carried out[27-29].

The type of dual-channel probe (sensors spacing 5, 10 or 15 cm) and its positioning in esophagus depended on the age of a patient (various lengths of esophagus) and clinical manifestation of GER (typical or atypical symptoms). The analysis of the type of reflux (acid/non-acid) was based on the pH monitoring values recorded in distal channel (2).

Recording from proximal channel (1) of the probe above LES (at various height) enabled the assessment of the range of reflux (high/low reflux) and the control of the proper positioning of the probe. It was also possible to compare the number of acid and non-acid refluxes in both positions of pH-monitoring. Esophageal pH monitoring always began in the morning, after fasting all night. The study lasted 24 h and comprised night sleep.

Patients discontinued particular medicines 3 d before the test. Among these medicines were: antacids, gastrokinetics, medications affecting tension of LES. Proton pomp inhibitors, H2-receptor antagonists were discontinued 7 d before the test[30].

Computer calculations of measurements obtained from both channels concerned the following pH-monitoring parameters: number of episodes of acid GER (intraesophageal pH below 4.0), number of episodes of acid GER lasting more than 5 min (so-called "long episodes"), reflux index i.e. the percentage of time that the pH is below 4.0. ESPGHAN diagnostic criteria were implemented in diagnosis of acid GER in examined children[14,15].

In children below 2 years of age the values of intraesophageal pH-monitoring were juxtaposed against normative values (borderline values) of Vandenplas *et al*[16,17] and another authors[18,19].

In older children (above 2 years of age) the borderline values at quantitative and qualitative assessment of pathological GER in both channels were[14,15,31-33]: total number of acid GER episodes (pH < 4.0/24 h) = 50; number of episodes of acid GER lasting more than 5 min ⩽ 2; the percentage of time with pH below 4.0 (%)-total acid GER index = 5.0%; the percentage of time with pH below 4.0 (%)-acid GER index supine = 2.5%.

Differentiation of pathological acid GER

In order to differentiate pathological GER into primary (idiopathic) and secondary - triggered off or aggravated by CMA/FA, the own diagnostic and therapeutic algorithm was administered in examined children. This algorithm comprises selected immunoallergological tests[10,23-25,34].

Skin tests (Prick): (1) with 12 native food allergens i.e. fresh (cow's milk, soya, of hen's egg white, hen's egg yolk, chicken's meat, beef, wheat flour, peanuts, bananas, fish, orange, white sesame); (2) with 9 commercial inhalant allergens (SmithKline Beecham-USA) (house dust mites, grass , trees, bushes and weeds pollens, dog's fur, cat's fur, mixed feathers, wool).

71 out of 138 children, of different age, with pathological acid GER and 32 children with CMA/FA exclusively, underwent these test once in order to confirm or exclude the ability of early IgE-dependent hypersensitivity to food allergens and/or inhalant allergens (atopic factor influence and or cross reactions) to trigger off symptoms observed. Results of control tests were the point of reference in assessment of reaction to allergens. The diameter of blister ⩾ 3 mm assessed after 15-20 min of allergen placement was concerned a positive result of skin Prick tests, compared to negative result of negative control.

Eosinophilia: One-time assessment of relative eosinophilia in full blood count and its analysis were performed in 138 children with pathological acid GER and in 32 children with CMA/FA exclusively. Improper percentage value of eosinophilia, determined in full blood count, was > 5%.

Total IgE concentration (c IgE) in serum-assessed with Fluoro-Fast method (3M Diagnostic Systems, USA): One-time assessment of serum IgE concentration was done in 170 children-138 with acid GER and 32 with CMA/FA exclusively. Serum c IgE concentration > 50 IU/mL was considered as elevated in examined children. Taking into cosideration restricted specificity of one-time measurement of total IgE in diagnosis of atopy, this test was performed together with determination of specific Ig in this particular class for selected food allergens.

Qualitative and quantitative assessment of specific IgE against food allergens (a-s IgE) and inhalant allergens (i-s IgE) with Fluro-Fast method (3M Diagnostic Systems, USA): Assay of allergen specific Ig concentration in examined children enabled confirmation of IgE-dependent pathomechanism of food allergy and determination of food allergens. These tests appeared to be helpful in cases where tests cannot be performed or their results are doubtful, due to various reasons. 103 patients suspected of allergy, with positive Prick tests results (food allergens and/or inhalant allergens and

increased total serum IgE concentration) underwent qualitative and quantitative assessment of a-s IgE and i-s IgE. Positive results of specific IgE were: a/a-s IgE against cow milk proteins, hen's egg white, hen's egg yolk, soy, fish, orange b/i-s IgE against grass, trees, bushes and weeds pollens, house dust mites and cat's fur, assayed in serum-presence supported in class ⩾ 2-5.

Oral food challenge test[25,26]: Open or blind oral food challenge test (depending on the age of patient) was carried out in order to establish causative relationship between food and clinical symptoms, regardless of pathogenetic mechanisms of allergy (IgE-dependent or IgE-non-dependent)[25]. The first stage of diagnostic procedure preceding the beginning of oral food challenge tests was eliminatory diet implementation, lasting 4 wk in 138 children with acid GER. Diet depended on elimination of the most common food allergens, suspected of triggering off symptoms in examined children. Eliminatory diet was determined on the basis of information gathered from medical history of past nutrition and the results of additional tests (skin Prick tests, s IgE)[25,26]. At the time of study, patients didn' t receive or had maximally reduced antillergic and/or antihistaminic medications. 138 children at various age, with pathological acid GER, after eliminatory diet implementation (milk-free and/or hypoallergic diet) underwent 204 biological oral food challenge tests; 138 (67.6%) with cow's milk and 66 (32.4%) with other food. In order to establish primary diagnosis, open food challenge test was performed in the 104 youngest children (below 3 years of age) and blind food challenge test in 34 children (above 3 years of age) with mainly cow's milk (low-lactose Bebilon, Ovita Nutricia) or with other potentially noxious nutrients[25,26]. Every time child spent 1-3 d at hospital (Laboratory of Allergy Diagnostics, of Ⅲ Department of Pediatrics). Time of appearance of biological reaction in examined child was counted from the last food challenge up to 48-72 h after intake of specific nutrient in native, blind form. Every patient examined received every day observation chart for reporting intensity of clinical manifestation. In case of cow's milk allergy or soy milk allergy and/or other food allergy the time of challenge test lasted 4 wk. Positive result of food challenge test and/or positive results of immunoallergological tests enabled to qualify a selected 62 children into group 2-children with GER secondary to FA.

In order to exclude the cause of secondary GER other than food allergy, the results of additional examinations performed in patients were analyzed. Among these examinations were: chest and upper gastroinestinal tract X-ray with barium swallow, X-ray or computed tomography of sinuses (in school children). On the basis of the aforementioned examinations, the type, localization and character of coexisting ailments were assessed. In order to confirm or exclude infectious cause of the symptoms presented, the following tests were taken into consideration: full blood count, erythrocyte sedimentation rate, CRP, ASO, protein fraction pattern, concentration of IgA, IgM, IgG, IgG antibodies against *Helicobacter pylori* and iron level. Moreover, bacteriological examinations were performed in some children (tests of blood, urine, faeces, bile, pharyngeal and nasal excretion). Pilocarpine test (chlorine concentration in perspiration) was performed to exclude cystic fibrosis. Moreover, metabolic screening was done by assaying lactic acid, ammonia, acid-base balance parameters in blood[2,9,13,34].

Table 1 Qualification of 264 children suspected of GERD into study groups (at diagnosis)

Groups of examined children	Sex	Examined children with reflux symptoms							
		Number		Age range (mo)					
				>1.5-4		>4-16		>16-102	
		n	%	*n*	%	*n*	%	*n*	%
Group 1 physiological	Boys	17	6.4	17	6.4	-	-	-	
GER *n* =32	Girls	15	5.7	15	5.7	-	-	-	-
Group 2	Boys	39	14.8	-	-	23	8.7	16	6.1
Primary GER *n* = 76	Girls	37	14	-	-	21	7.9	16	6.1
Group 3	Boys	33	12.5	-	-	16	6.1	17	6.4
GER + CMA/FA *n* = 62	Girls	29	11	-	-	14	5.3	15	5.7
Group 4 reference group	Boys	19	7.2	-	-	7	2.6	12	4.5
CMA/ FA *n* = 32	Girls	13	4.9	-	-	5	1.9	8	3
Group 5 GER (-) + CMA/	Boys	32	12.1	-	-	8	3	24	9.1
FA (-) *n* = 62	Girls	30	11.4	-	-	10	3.8	20	7.6
Total		264	100	32	12.1	104	39.4	128	48.5

Assignment of children into study groups

264 children were assigned into specific study groups (Table 1) after consideration of the results of 24-h esophageal pH monitoring, complex differential diagnosis, oral food challenge test with noxious nutrient, eliminatory diet, and nutrition analysis.

Acid GER was diagnosed in 170 children. Out of 170 patients (64.4%) with acid GER, of both sexes i.e. 89 boys and 81 girls, 32 (12.1) infants with physiological GER (group 1) were selected. This selected group consisted of 17 boys (6.4%) and 15 girls (5.7%), aged 1.5-4 mo (mean age $\chi = 2.2 \pm 0.48$ mo).

The diagnosis was put forward on the basis of the number of reflux episodes exclusively, recorded during pH monitoring. The results of remaining parameters were within the normative reference values (age-related normative values). Due to physiological character of reflux (not complicated), typical for the youngest patients, these infants were not the subject of prospective clinical observation and further clinical analysis.

In 138 children (52.3%) pathological acid GER was diagnosed and classified into primary and secondary GER. These children were assigned into group 2 and 3. Group 2 constituted 76 patients (28.8%) with pathological primary acid GER, of both sexes (39 boys-14.8%, 37 girls-14.0%), aged 4-102 mo (mean age $\chi = 25.2 \pm 27.28$ mo). In group 3 were 62 patients (23.5%), of both sexes (33 boys-12.5%; 29 girls-11.0%), aged 74 mo (mean age $\chi = 21.53 \pm 17.79$ mo) with pathological GER secondary to CMA/FA.

Acid GER was not confirmed in 94 (35.6%) out of 264 patients with symptoms suggesting GERD. These children were qualified into groups 4 and 5. Group 4-the reference group constituted 32 patients (12.1%), of both sexes (19 boys-7.2%;13 girls-4.9%), aged 7-69 mo (mean age $\chi = 23.7$

Table 2 Statistically analysis of selected parameters of 24-h pH-monitoring in 264 children suspected of GERD; pH-monitoring with 1- or 2- channel probe

Groups of examined children with specific ailment	pH-monitoring parameters-distal channel range of values; mean; standard deviation (± SD); median; statistical significance (*P*)				
n = 264	Number of episodes of acid GER (pH < 4)	Number of episodes of acid GER (pH < 4), lasting > 5 min	Duration of the longest episode of acid GER (min)	Total acid GER index (%)	Acid GER index (supine position) (%)
	Statistical significance between the groups (*P*)				
Group 2 and 3	*P* = 0.0001	*P* = 0.0001	*P* = 0.0001	*P* = 0.0001	NS
Group 2 and 4				*P* = 0.0001	*P* = 0.0001
Group 2 and 5				*P* = 0.0001	*P* = 0.0001
Group 3 and 4				*P* = 0.0001	*P* = 0.0001
Group 3 and 5				*P* = 0.0001	*P* = 0.0001
Group 4 and 5				NS	NS

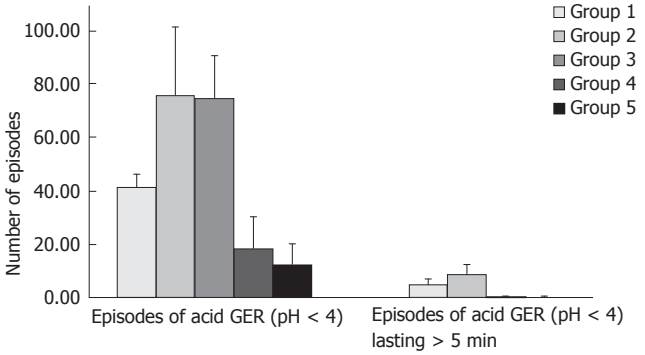


Figure 1 Summary analysis of number of episodes of acid GER (pH < 4) and episodes of acid GER (pH < 4) lasting > 5 min of 24-h pH-monitoring in 264 children suspected of GERD; pH-monitoring with 1[1]- or 2-channel probe. [1]Values of pH-monitoring parameters in group 1 did not undergo comparative analysis with corresponding values in remaining groups.

± 12.63 mo) with symptoms typical for cow milk allergy and/or other food allergy (CMA/FA). Group 5 constituted 62 patients (23.5%) (32 boys-12.1%; 30 girls-11.4%), aged 4-102 mo (mean age χ = 31.3 ± 27.98 mo).

Neither reflux cause nor allergic cause of the symptoms were confirmed in these children, and hence they were not the subject of prospective observation and further clinical analysis.

The study was approved by local Bioethical Committee of the Medical University of Bialystok and informed parental consent was obtained from parents of examined children.

Statistical analysis

The statistical analysis of the results comprised arithmetical mean, standard deviation, minimal and maximal values and median-for measurable features and quantitative percentage distribution for qualitative features. To compare the groups, features compatible with normal distribution, assessed with Kolomogorov compatibility test, were assessed together with the post hoc Bonferroni one-way analysis of variance. Features non-compatible with the distribution underwent Kruskal-Wallis test and if the differences were statistically significant, Mann-Whitney test was applied. Statistical significance was $P < 0.05$. Calculations were performed with the help of statistical package SPSS'12.0 PL.

RESULTS

24-h pH monitoring

Preliminary comparative analysis of selected values of 24-h intraesophageal pH monitoring parameters with single-channel probe (distal channel) was carried out in 32 children (12.1%) (group 1) and with dual-channel (distal and proximal channel) in 232 children (87.9%) (groups 2, 3, 4 and 5). The results of the analysis are presented in Tables 2, 3 and Figures 1-6 Results of pH esophageal monitoring in group 1 did not undergo comparative analysis with respective values obtained in children from remaining groups. Pathological acid GER was diagnosed in 138 children (52.3%), in 76 children primary GER (group 2) and in 62 children GER secondary to CMA/FA (group 3). Pathological acid GER was not confirmed in 94 children (35.6%), out of which 32 had CMA/FA diagnosed (group 4-reference group). In remaining 62 children neither GER nor CMA/FA were confirmed (group 5). The values of esophageal pH monitoring parameters obtained in individual groups: 2, 3, 4, and 5 were compared against each other. The analysis of mean values of pH monitoring parameters measured in children from individual groups is presented as follows.

According to the number of episodes of acid GER (pH < 4)

Distal channel (Table 2, Figure 1): In children with primary GER (group 2) the mean value of the parameter (χ = 75.68 ± 25.61) was similar to mean value (χ = 74.6 ± 16.02) in children with GER and CMA/FA (group 3). These values are higher than mean number of episodes of acid GER, constituting χ = 18.56 ± 11.74 and χ = 12.6 ± 7.74 in group 4 and 5, respectively.

Proximal channel (Table 3, Figure 2): In children from group 2 mean value of the examined parameter (χ = 61.45 ± 20.43) was similar to mean value (χ = 62.48 ± 14.67) in children from group 3. These values are higher than mean number of episodes of acid GER χ = 10.5 ± 4.5 and χ = 5.36 ± 2.09 in group 4 and group 5, respectively.

According to number of episodes of acid GER lasting > 5 min

Distal channel (Table 2, Figure 1): In children from group 2 mean value of this parameter (χ = 5.17 ± 1.95) was lower than mean value (χ = 8.87 ± 3.65) in children from group 3. At the same time these values are higher

Table 3 Summary analysis of selected parameters of 24-h pH-monitoring in 232 children suspected of GERD; pH-monitoring with 2-channel probe

Groups of examined children with specific ailment	pH-monitoring parameters-proximal channel range of values; mean; standard deviation (± SD); median; statistical significance (*P*)				
n = 232	Number of episodes of acid GER (pH < 4)	Number of episodes of acid GER (pH < 4), lasting > 5 min	Duration of the longest episode of acid GER (min)	Total acid GER index (%)	Acid GER index (supine position) (%)
	Statistical significance between the groups (*P*)				
Group 2 and 3 Group 2 and 4 Group 2 and 5 Group 3 and 4 Group 3 and 5 Group 4 and 5	*P* = 0.0001	*P* = 0.0001	*P* = 0.0001	*P* = 0.0001	*P* = 0.0001

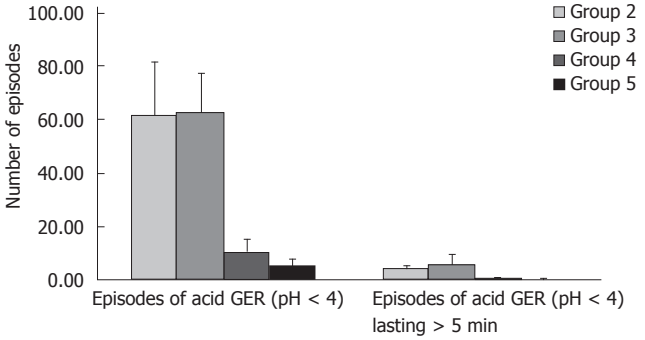


Figure 2 Summary analysis of number of episodes of acid GER (pH < 4) and episodes of acid GER (pH < 4) lasting > 5 min of 24-h pH-monitoring in 232 children suspected of GERD; pH-monitoring with 2- channel probe.

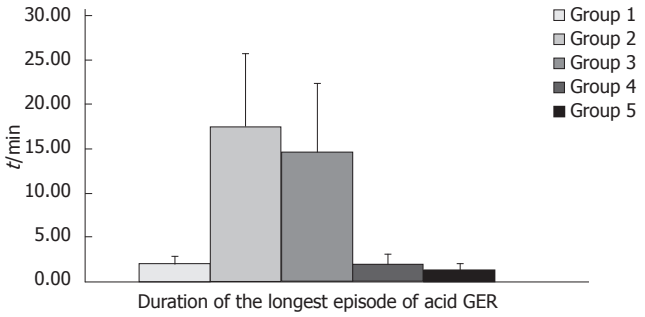


Figure 3 Summary analysis of duration of the longest episode of acid GER of 24-h pH-monitoring in 264 children suspected of GERD; pH-monitoring with 1[1]- or 2- channel probe. [1]Values of pH-monitoring parameters in group 1 did not undergo comparative analysis with corresponding values in remaining groups.

than mean values of parameters $\chi = 0.33 \pm 0.49$ and $\chi = 0.15 \pm 0.36$ measured in group 4 and 5, respectively.

Proximal channel (Table 3, Figure 2): In children from group 2 mean value of parameter measured ($\chi = 3.96 \pm 1.37$) was lower than mean value in group 3 ($\chi = 5.87 \pm 3.64$). At the same time these values are higher than mean values of episodes of acid GER lasting more than 5 min: $\chi = 0.28 \pm 0.46$ and $\chi = 0.11 \pm 0.32$ in group 4 and 5, respectively.

According to the duration of the longest episode of acid GER (minutes)

Distal channel (Table 2, Figure 3): In children from

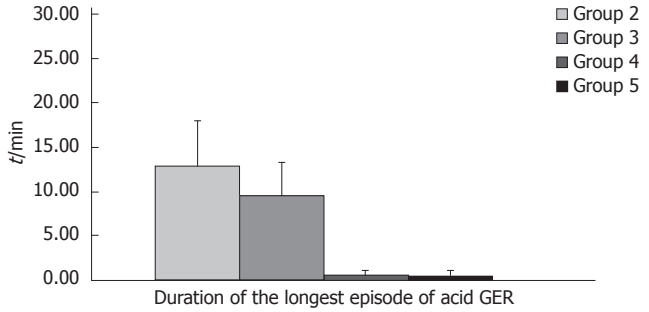


Figure 4 Summary analysis of duration of the longest episode of acid GER of 24-h pH-monitoring in 232 children suspected of GERD; pH-monitoring with 2-channel probe.

group 2 mean value of this parameter ($\chi = 17.45 \pm 8.21$) was higher than mean value ($\chi = 14.61 \pm 7.68$) in children from group 3. These values were higher than mean time of the longest episode of acid GER: $\chi = 2.08 \pm 1.05$ and $\chi = 1.4 \pm 0.66$ in group 4 and 5, respectively.

Proximal channel (Table 3, Figure 4): In children from group 2 mean value of parameter measured ($\chi = 12.91 \pm 5.14$) was higher than mean value ($\chi = 9.51 \pm 3.78$) in children from group 3. At the same time these values are higher than mean time of the longest episode of acid GER: $\chi = 0.67 \pm 0.49$ and $\chi = 0.44 \pm 0.62$ in group 4 and 5, respectively.

According to total acid GER index (%)

Distal channel (Table 2, Figure 5): In children from group 2 mean value of this parameter ($\chi = 13.42 \pm 5.52$) was lower than mean value ($\chi = 17.17 \pm 6.96$) in children from group 3. These values are higher than mean values of total acid GER index: $\chi = 2.69 \pm 1.07$ and $\chi = 3.1 \pm 0.78$ in groups 4 and 5, respectively.

Proximal channel (Table 3, Figure 6): In children from group 2 mean value of this parameter ($\chi = 11.26 \pm 4.18$) was higher than mean value ($\chi = 10.47 \pm 3.8$) in children from group 3. At the same time these values were higher than mean values of total acid GER index: $\chi = 0.91 \pm 0.68$ and $\chi = 0.37 \pm 0.16$ in group 4 and 5, respectively.

According to acid GER index, supine position (%)

Distal channel (Table 2, Figure 5): In children from

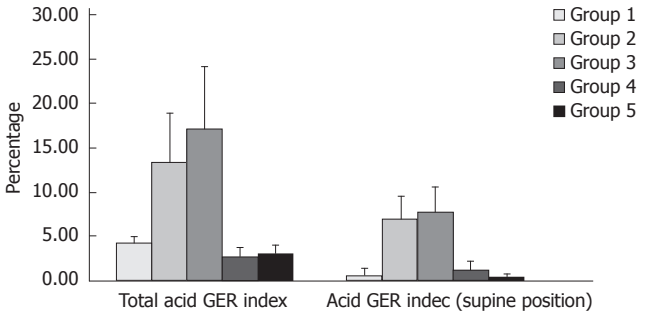


Figure 5 Summary analysis of total acid GER index and acid GER index (supine position) of 24-h pH-monitoring in 264 children suspected of GERD; pH-monitoring with 1[1]- or 2-channel probe. [1]Values of pH-monitoring parameters in group 1 did not undergo comparative analysis with corresponding values in remaining groups.

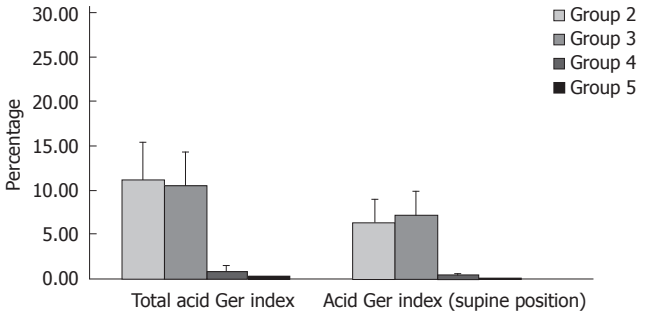


Figure 6 Summary analysis of total acid GER index and acid GER index (supine position) of 24-h pH-monitoring in 232 children suspected of GERD; pH-monitoring with 2- channel probe.

group 2 mean value of this parameter (χ = 6.96 ± 2.64) was lower than mean value (χ = 7.67 ± 2.87) in children from group 3. These values were higher than mean values of supine acid GER index: χ = 1.09 ± 1.06 and χ = 0.39 ± 0.31 in groups 4 and 5, respectively.

Proximal channel (Table 3, Figure 6): In children from group 2 mean value mean value of parameter measured (χ = 6.41 ± 2.64) was lower than mean value (χ = 7.16 ± 2.76) in children from group 3. These values were higher than mean value of supine acid GER index: χ = 0.43 ± 0.22 and χ = 0.09 ± 0.11 in groups 4 and 5, respectively.

The comparative analysis carried out between group 2 and 3 (children with GER) and between group 4 and 5 (children without GER) proved statistically significant differentiation ($P < 0.05$) of mean values (abnormal distribution) in the case of: number of episodes of acid GER, number of episodes of acid GER lasting more than 5 min and duration of the longest episode of acid GER in both channels (distal and proximal) as well as acid GER index: total and supine, in proximal channel exclusively.

This analysis also confirmed statistically significant differences ($P < 0.05$) of mean values (normal distribution) in the case of total acid GER index, in distal channel exclusively, among individual groups, especially between group 2 (children with primary GER) and group 3 (children with GER secondary to CMA/FA). However, in the case of supine acid GER, in distal channel, no statistical significance of its mean values between group 2 and group 3 was confirmed.

Table 4 Type of graphic recording of intraesophageal pH in 264 examined children suspected of GERD at diagnosis

Groups of examined children	24-h intraesophageal pH-monitoring - preliminary study							
	Pathological recording				Regular recording (physiological)			
	Phasic		Non phasic		Phasic		Non phasic	
	n	%	*n*	%	*n*	%	*n*	%
Group 1[1] *Physiological GER* *n* = 32 (100.0%)	-	-	-	-	-	-	32	100
Group 2 Primary GER *n* = 76 (100.0%)	-	-	76	100	-	-	-	-
Group 3 GER + CMA/FA *n* = 62 (100.0%)	9	14.5	53	85.5	-	-	-	-
Group 4 reference group *n* = 32 (100.0%)	-	-	-	-	3	9.4	29	90.6
Group 5[1] GER (-) + CMA/FA (-) *n* = 62 (100.0%)	-	-	-	-	-	-	62	100

[1]Children excluded from further prospective clinical studies.

On the basis of pH monitoring recording at preliminary examination in children of individual groups (Table 4), phasic recording of intraesophageal pH monitoring values was registered in 9 children (14.5%) with pathological GER secondary to CMA/FA (group 3) and in 3 children (9.4%) with CMA/FA exclusively (group 4-reference group).

DISCUSSION

Of 264 examined children, acid GER was confirmed in 170 (64.4%) of them on the basis of 24-h esophageal pH monitoring. In 138 of these children (52.3%) GERD was confirmed.

Age of examined children did not reveal statistically significant differences among individual groups. Among 138 children, GERD was more often attributed to acid GER in boys (72-52.2%) in comparison to girls (66%-47.8%).

On the basis of the results of immunoallergological tests and verifying positive oral food challenge test[25,26], pathological acid GER in 138 children was divided into primary (28.8% of children, group 2) and secondary to CMA/FA (23.5%, group 3). At the same time, on the basis of complex differential diagnosis other causes of acid GER were excluded.

Among 264 children with family history of gastrointestinal tract diseases, acid GER was not confirmed in 94 of them (35.6%).

The assumption of esophageal pH monitoring was that the higher the electrode sensor was positioned, the less number of short-term reflux episodes was recorded, and the total time of reflux is shortened, which is consequent upon better efficiency of neutralizing mechanisms and the ability to self-purification of this part of esophagus[18,20,32,33].

The results of our studies do not support the stated hypothesis completely because the mean values of analyzed

pH monitoring parameters in proximal channel were not lower (not statistically significant) than in distal part of esophagus in children with GERD in groups 2 and 3.

Percentage values of the number of episodes of acid GER recorded in proximal channel accounted for 81%, in group 2 and 84% in distal channel, in group 3.

The number of episodes of acid GER lasting more than 5 min recorded in proximal part of esophagus accounted for 76% in group 2, whereas in distal part of esophagus made 66%, group 3.

The duration of the longest episode of acid GER recorded in proximal part of esophagus constituted 74%, group 2, whereas in distal part of esophagus it accounted for 65%, group 3. Total acid GER index recorded in proximal part of esophagus made 84%, group 2 and in distal part of esophagus 61%, group 3. Supine acid GER index in proximal part of esophagus accounted for 92%, group 2 and in distal part of esophagus it made 93%, group 3.

In children with primary GER (group 2) and secondary GER (group 3), the mean values of individual pH monitoring parameters did not reveal significant difference between both channels. On the basis of the results obtained, it appears that there was no significant quantitative difference in the episodes of acid GER, reaching both distal and proximal channel, regardless of the age of examined children.

It was shown that reflux episodes in proximal channel were similar in number only in patients with primary GER (group 2) and those with GER secondary to CMA/FA (group 3). It may be assumed that high gastroesophageal reflux reaching proximal channel is particularly meaningful in children of both groups, but with atypical symptoms, especially of respiratory tract (silent reflux), therefore suggesting the possibility of microaspiration of gastric content into the bronchial tree[2,18-20].

Silent reflux in children below 3 years of age, with recurrent infections of respiratory tract in past history was confirmed on the basis of pH monitoring with single-channel probe in 56% and 57% of children's gastroenterological centers in Poland[35,36].

According to these data the diagnostic value (sensitivity) of 24-h esophageal pH monitoring in detecting pathological GER accounted for 89% in all patients examined with this type of probe but made only 84% in patients with atypical symptoms.

On the basis of 24-h esophageal pH monitoring with dual-channel probe in children with symptoms out of gastrointestinal tract, within the same age group, in own studies the higher percentage of high gastroesophageal reflux was reported, which accounted for 77.4% and 88.3% in both groups, respectively. The results of own studies give information on the intensity of acid GER reaching distal and proximal part of esophagus, and mean values of examined pH monitoring parameters in distal and proximal part of esophagus reveal statistical significance between the groups. The comparable results of supine acid GER index in distal channel constitute an exception. This differentiation of pH monitoring parameters between the groups appears to be helpful in predicting, who of the examined children is at risk of primary GER, and which symptoms are consequent upon GER secondary to CMA/FA. The result of preliminary study s also important, in which the increasing number of reflux episodes reflecting the higher value of reflux index, and it was comparable in distal and proximal channel, in both study groups.

Italian authors reported the interpretation of graphic recording of intraesophageal pH monitoring, in which they showed a phasic decrease of values after milk meal and their rapid increase after the following meal in children with GER and/or CMA[37].

Quantitative characteristics of patients of individual groups proved that this phasic recording of pH was a common feature in 9 children (14.5%) with GER and CMA/FA (group 3) and in 3 children (9.4%) with CMA/FA (group 4-reference group), which accounted for 12 out of 94 children with cow milk allergy. Interestingly enough, this type of recording of pH monitoring was not observed in any of the children with GER but without allergy.

In conclusion, pH monitoring performed in children with typical and atypical GERD symptoms enabled to diagnose acid GER in 52.3% of all examined patients. In order to define the extent of reflux i.e. the dynamics of each reflux episode, dual-channel probe should be used to intraesophageal pH recording in distal and proximal channel. Finally, similar pH monitoring results obtained in both study groups with GERD confirm that pH monitoring is clinically important for diagnosing of acid GER but not for differential diagnosis for between primary and secondary GER patients. This support the necessity of early implementation of complex differential diagnosis that enables to distinguish the causes of primary GER from the causes of GER secondary to FA. This causal differentiation leads to necessity of defining proper treatment strategy. It also influences the efficacy of treatment and natural history of gastroesophageal reflux disease in children and the young.

REFERENCES

1 **Shepherd RW,** Wren J, Evans S, Lander M, Ong TH. Gastroesophageal reflux in children. Clinical profile, course and outcome with active therapy in 126 cases. *Clin Pediatr* (Phila) 1987; **26**: 55-60

2 **Semeniuk J**. Ethiopathogenic role of gastro-oesophageal reflux in developing of clinical symptoms in children [dissertation]. Medical University of Białystok, 1990: 3

3 **Herbst JJ**. Gastroesophageal reflux. *J Pediatr* 1981; **98**: 859-870

4 **Nelson HS**. Gastroesophageal reflux and pulmonary disease. *J Allergy Clin Immunol* 1984; **73**: 547-556

5 **Herbst JJ**, Book LS, Bray PF. Gastroesophageal reflux in the "near miss" sudden infant death syndrome. *J Pediatr* 1978; **92**: 73-75

6 **Hermier M**, Descos B. Gastroesophageal reflux and respiratory manifestations. *Pediatrie* 1983; **38**: 125-135

7 **Malfertheiner P**, Hallerbäck B. Clinical manifestations and complications of gastroesophageal reflux disease (GERD). *Int J Clin Pract* 2005, **59**: 346-355

8 **Semeniuk J**, Kaczmarski M. Gastroesophageal reflux in children and adolescents. clinical aspects with special respect to food hypersensitivity. *Adv Med Sci* 2006; **51**: 327-335

9 **Semeniuk J**, Wasilewska J, Kaczmarski M, Lebensztejn D. Non-typical manifestation of gastroesophageal reflux in children. *Med Sci Monit* 1998; **4**: 1122-1130

10 **Iacono G**, Carroccio A, Cavataio F, Montalto G, Kazmierska I, Lorello D, Soresi M, Notarbartolo A. Gastroesophageal reflux and cow's milk allergy in infants: a prospective study. *J Allergy*

Clin Immunol 1996; **97**: 822-827
11 **Milocco C**, Torre G, Ventura A. Gastro-oesophageal reflux and cows' milk protein allergy. *Arch Dis Child* 1997; **77**: 183-184
12 **Semeniuk J**, Kaczmarski M, Nowowiejska B, Białokoz I, Lebensztejn D. Food allergy as the causa of gastroesophageal reflux in the youngest children. *Pediatr Pol* 2000; **10**: 793-802
13 **Salvatore S**, Vandenplas Y. Gastroesophageal reflux and cow milk allergy: is there a link? *Pediatrics* 2002; **110**: 972-984
14 A standardized protocol for the methodology of esophageal pH monitoring and interpretation of the data for the diagnosis of gastroesophageal reflux. Working Group of the European Society of Pediatric Gastroenterology and Nutrition. *J Pediatr Gastroenterol Nutr* 1992; **14**: 467-471
15 **Vandenplas Y**, Loeb H. The interpretation of oesophageal pH monitoring data. *Eur J Pediatr* 1990; **149**: 598-602
16 **Vandenplas Y**, Goyvaerts H, Helven R, Sacre L. Gastroesophageal reflux, as measured by 24-hour pH monitoring, in 509 healthy infants screened for risk of sudden infant death syndrome. *Pediatrics* 1991; **88**: 834-840
17 **Vandenplas Y**, Sacré-Smits L. Continuous 24-hour esophageal pH monitoring in 285 asymptomatic infants 0-15 months old. *J Pediatr Gastroenterol Nutr* 1987; **6**: 220-224
18 **Arana A**, Bagucka B, Hauser B, Hegar B, Urbain D, Kaufman L, Vandenplas Y. PH monitoring in the distal and proximal esophagus in symptomatic infants. *J Pediatr Gastroenterol Nutr* 2001; **32**: 259-264
19 **Bagucka B**, Badriul H, Vandemaele K, Troch E, Vandenplas Y. Normal ranges of continuous pH monitoring in the proximal esophagus. *J Pediatr Gastroenterol Nutr* 2000; **31**: 244-247
20 **Semeniuk J**, Kaczmarski M, Krasnow A, Sidor K, Matuszewska E, Daniluk U. Dual simultaneous esophageal pH monitoring in infants with gastroesophageal reflux. *Pol Merkur Lekarski* 2003; **14**: 405-409
21 **Kaczmarski M**. Food allergy and intolerance. Milk, sugars, soya. Sanmedia, Warszawa, 1993: 7-10
22 **Staiano A**, Troncone R, Simeone D, Mayer M, Finelli E, Cella A, Auricchio S. Differentiation of cows' milk intolerance and gastro-oesophageal reflux. *Arch Dis Child* 1995; **73**: 439-442
23 **Iacono G**, Carroccio A, Cavataio F, Montalto G, Lorello D, Kazmierska I, Soresi M. IgG anti-betalactoglobulin (betalactotest): its usefulness in the diagnosis of cow's milk allergy. *Ital J Gastroenterol* 1995; **27**: 355-360
24 **Cavataio F**, Iacono G, Montalto G, Soresi M, Tumminello M, Campagna P, Notarbartolo A, Carroccio A. Gastroesophageal reflux associated with cow's milk allergy in infants: which diagnostic examinations are useful? *Am J Gastroenterol* 1996; **91**: 1215-1220
25 **Kaczmarski M**. The stand of Polish Group of experts to food allergy and intolerance. Polish Society for Alergology, Symposium 1, Medical Convention Periodical, Unimed, 1997; **1**: 21-31, 39-67
26 **Matuszewska E**, Kaczmarski M, Semeniuk J. Oral challenge tests in diagnostics of ford allergy and intolerance. *Ped Współczesna Gastroenterol Hepatol i Żywienie Dziecka* 2000; **2-4**: 239-243
27 **Strobel CT**, Byrne WJ, Ament ME, Euler AR. Correlation of esophageal lengths in children with height: application to the Tuttle test without prior esophageal manometry. *J Pediatr* 1979; **94**: 81-84
28 **McCauley RG**, Darling DB, Leonidas JC, Schwartz AM. Gastroesophageal reflux in infants and children: a useful classification and reliable physiologic technique for its demonstration. *AJR Am J Roentgenol* 1978; **130**: 47-50
29 **Thor P**, Herman R, Plebankiewicz S, Bogdał J. Esophageal manometry and pH-metry in gastroesophageal reflux disease; their role in preoperative evaluation. *Acta Endosc Pol* 1994; **6**: 167-173
30 **Kiljander TO**, Laitinen JO. The prevalence of gastroesophageal reflux disease in adult asthmatics. *Chest* 2004; **126**: 1490-1494
31 **Gustafsson PM**, Tibbling L. 24-hour oesophageal two-level pH monitoring in healthy children and adolescents. *Scand J Gastroenterol* 1988; **23**: 91-94
32 **Cucchiara S**, Santamaria F, Minella R, Alfieri E, Scoppa A, Calabrese F, Franco MT, Rea B, Salvia G. Simultaneous prolonged recordings of proximal and distal intraesophageal pH in children with gastroesophageal reflux disease and respiratory symptoms. *Am J Gastroenterol* 1995; **90**: 1791-1796
33 **Little JP**, Matthews BL, Glock MS, Koufman JA, Reboussin DM, Loughlin CJ, McGuirt WF. Extraesophageal pediatric reflux: 24-hour double-probe pH monitoring of 222 children. *Ann Otol Rhinol Laryngol Suppl* 1997; **169**: 1-16
34 **Semeniuk J**, Tryniszewska E, Wasilewska J, Kaczmarski M. Food allergy- causal factor of gastroesophageal reflux in children. *Terapia* 1998; **6**: 16-19
35 **Fyderek K**, Stopyrowa J, Sładek M. Gastroesophageal reflux as a risk factor in different diseases in children. *Przegl Lek* 1991; **48**: 385-389
36 **Zielińska I**, Czerwionka-Szaflarska M. The value of pH-metric examination in diagnostics of recurrent bronchitis and pneumonia. *Przegl Pediatr* 1999; **supp l**: 52-54
37 **Cavataio F**, Iacono G, Montalto G, Soresi M, Tumminello M, Carroccio A. Clinical and pH-metric characteristics of gastro-oesophageal reflux secondary to cows' milk protein allergy. *Arch Dis Child* 1996; **75**: 51-56

S- Editor Liu Y **L- Editor** Rampone B **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5116-5120
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Crohn's disease incidence evolution in North-western Greece is not associated with alteration of NOD2/CARD15 variants

Michael Economou, Grigoris Filis, Zoi Tsianou, John Alamanos, Antonios Kogevinas, Kostas Masalas, Anna Petrou, Epameinondas V Tsianos

Michael Economou, Grigoris Filis, Zoi Tsianou, Antonios Kogevinas, Kostas Masalas, Anna Petrou, Epameinondas V Tsianos, 1st Department of Internal Medicine and Hepato-gastroenterology Unit, University of Ioannina, School of Medicine, Ioannina, Greece
John Alamanos, Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece
Supported by General Secretariat for Research and Technology, Greece and the European Union, PENED03ED770
Correspondence to: Michael Economou, 1st Department of Internal Medicine and Hepato-gastroenterology Unit, University of Ioannina School of Medicine, Ioannina 45110, Greece. meconom@cc.uoi.gr
Telephone: +30-265-1097799 Fax: +30-265-1097867
Received: March 16, 2007 Revised: April 7, 2007

Abstract

AIM: To assess the trends in the incidence of inflammatory bowel disease (IBD) over 23 years in the same area and to identify genetic factors related to incidence evolution.

METHODS: Patients with IBD arising from North-western Greece were systematically recorded through the 1983-2005 period. Trends in disease incidence and genetic patterns related to CARD15 variants were documented and correlated.

RESULTS: A total of 447 patients with IBD were recorded (23.5% Crohn's disease, 72.7% Ulcerative colitis and 3.8% indeterminate colitis). Mean annual incidence rates of CD and UC were 0.9/100 000 (95% CI 0.1-1.7) and 2.7/100 000 (95% CI 1.7-4.1) inhabitants, respectively. There was a statistically significant increase of CD incidence ($P < 0.01$) during the study period, in contrast to the UC incidence. There were no statistical differences in CARD15 variants over the study period.

CONCLUSION: The incidence of CD in North-western Greece has risen disproportionately to that of UC in the 21st century. This is not related to alterations of genetic background though.

Key words: Inflammatory bowel disease; Crohn's disease; Epidemiology; CARD15

Economou M, Filis G, Tsianou Z, Alamanos J, Kogevinas A, Masalas K, Petrou A, Tsianos EV. Crohn's disease incidence evolution is not associated with alteration of NOD2/CARD15 variants. *World J Gastroenterol* 2007; 13(38): 5116-5120

http://www.wjgnet.com/1007-9327/13/5116.asp

INTRODUCTION

Inflammatory bowel disease (IBD) encompasses two distinct clinicopathological entities, Crohn's disease (CD) and ulcerative colitis (UC), possessing different etiological and epidemiological correlations. Both are considered of unknown aetiology, and various endogenous and exogenous factors have been etiologically implicated, leading to the concept of a multifactorial pathogenetic process involving interplay of environmental risk factors and immunologically triggered alterations[1]. Environmental factors have for long been incriminated in the pathogenesis of both forms of IBD, usually based on data of geographic or temporal variation of disease incidence. A global north-south variation in the incidence of IBD has been reported[2]. In Europe, the EC-IBD population based study showed a higher incident of UC (11.8/100 000 *vs* 8.7/100 000) and CD (7.0/100 000 and 3.9/100 000, respectively) in Northern compared to Southern Europe[3]. Such variations do exist in the overall prevalence of each form, with variable data on the incidence trends of CD in recent years[4-6], but an invariable increase in CD incidence in Southern European regions[7,8]. Evaluation of the factors triggering this evolving incidence may allow for identifying major endogenous or exogenous factors related to the pathogenesis of the disease.

In North-western Greece, an area traditionally categorized to the poorest of Europe, previous studies have shown rarity of CD and common appearance of UC during the previous decades[9,10]. This study assesses current evolution of incidence of CD and correlates these trends with genetic (CARD15 mutations) factors.

MATERIALS AND METHODS

Study area

The study area, North-western Greece, represents a population of 506 142 inhabitants according to the

National Census of 2001, including four Districts situated on the mainland (Districts of Ioannina, Arta, Preveza and Thesprotia) and two Districts situated in islands (Districts of Corfu and Lefkada). Urban residents represented about 40% of the total population, residing mainly in the District capitals.

Case identification and diagnostic criteria

Cases have been recorded in the frame of a systematic recording system for IBD, using multiple sources of retrieval, developed in this defined area of North-western Greece between January 1983 and December 2005. The health care system in our area includes both a National Health Service and a private sector. All gastroenterologists are members of North-western Greece Gastroenterology Group and were updated about the methods and aims of this study both through newsletters and meetings. The system records cases from the following sources: (1) in- and outpatients referred to the Hepato-gastroenterology Unit of the Ioannina University Hospital; (2) patients referred to outpatient Gastroenterology clinics in Districts hospitals and (3) patients referred to the private gastroenterologists practicing inside the area. A review of collected data was performed in the University Hospital of Ioannina, by two investigators (ME and EVT). Diagnosis of CD and UC was based on typical clinical, radiological, endoscopic and histological criteria and the diagnosis of intermediate colitis (IC) was only set when a distinct diagnosis of UC or CD could not be established. CD was classified according to the Vienna System[11] and UC according to Lennard-Jones[12]. Perianal disease was defined by the presence of perianal abscesses, fistulae or ulcers, but not by the presence of skin tags. A final diagnosis of CD, UC, or ulcerative proctitis (UP) though was made by two expert gastroenterologists and recorded as definite, probable, or possible following criteria previously published[9]. Phenotypic details were obtained on 2 occasions by retrospective case-note review between January and December 2005 by 2 investigators (ME and EVT). Duration of follow-up was defined as the interval between diagnosis and latest case-note review. Details regarding other demographic characteristics were further supplemented by a patient interview or completed postal questionnaire. During the last year blood was collected from all CD patients. An incidence case was defined as any IBD patient, diagnosed during the study period, resident in the study area for at least one year before the diagnosis. All patients first diagnosed during the study period 1983-2005 were included in the study. All patients included in the study were evaluated at least twice during the study period.

The study protocol conformed to the ethical guidelines of the Declaration of Helsinki.

Genotyping

Three NOD2/CARD15 variants previously identified as being independently associated with Crohn's disease, R702W, G908R, L1007fsinsC[13-15] were genotyped. Each individual reaction contained primers to amplify a nonpolymorphic genomic control sequence. A single thermocycling program was used for all reactions. The products were electrophorerized on 1% agarose gels with ethidium bromide and viewed under ultraviolet light. An image was recorded digitally. Healthy controls match for age and gender with CD patients were randomly selected from Blood Donors in University Hospital of Ioannina and District Hospital of Corfu.

Table 1 Demographic characteristics of IBD patients diagnosed during the period 1983-2005, in northwest Greece

	Crohn's disease	Ulcerative colitis
Total numbers of patients	105	325
Region of origin		
Ioannina	59	140
Arta	5	10
Preveza	2	36
Thesprotia	5	21
Corfu	33	113
Lefkada	1	5
Male: female (ratio)	66:39 (1.69)	208:117 (1.77)
Age at diagnosis (yr) (mean ± SD)	33.2 ± 12.9	45.7 ± 16.1
(range)	(18-70)	(18-93)
Men	33.4 ± 12.9	47.3 ± 15.1
Women	33.1 ± 13.2	42.9 ± 17.3
Follow up (mo) (mean ± SD)	92.9 ± 72.3	119.6 ± 73.8
(range)	(4-264)	(5-276)

Statistical analysis

Incidence rates were calculated as the number of cases per 100 000 inhabitants, and 95% Confidence Intervals were estimated using the normal distribution. Population data were based on databases of the National Statistical Service (National Censuses 1981, 1991, and 2001). The significance of time trends of incidence rates was tested using the χ^2-trends in proportion test. Frequency and susceptibilities of NOD2/CARD15 variants among CD patients, UC patients and controls was compared with the chi-square test. Odds ratios (OR) were calculated with the chi-squared distribution test and 95% confidence intervals (95% CI). The Fisher exact test was used for comparing differences between allele frequencies in patients and controls. The *P* values obtained were two-tailed and statistical significance was assumed below 0.01. Inference was aided by GraphPad InStat (version 3.00; GraphPad Software).

RESULTS

During the study period, a total of 447 IBD cases were recorded in the study area. One hundred and five patients were diagnosed with CD (23.5%), 325 with UC (72.7%), and 17 with Indeterminate Colitis (3.8%). The majority of the patients were derived from the regions of Ioannina and Corfu, accounting for 92% of the total CD cases and 78% of the total UC cases.

The main demographic and clinical characteristics of CD and UC patients are presented in Table 1.

A familial history of IBD was present in 11 patients (2.5%). Terminal ileal localization of the disease was present in 35 (33.3%), ileocolonic in 28 (26.7%) and colonic in 42 (40%) of the CD patients; stenotic disease

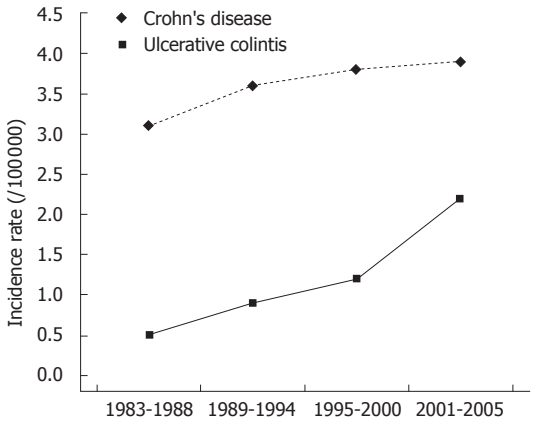


Figure 1 Trends in standardised incidence of Crohn's disease (CD) and Ulcerative colitis (UC) (Northwestern Greece 1983-2005).

Table 2 Evolution of genetic, demographic and clinical correlations of Crohn's disease through the study period

Period	Any CARD15 mutation	M:F ratio	Mean age (yr)	CD:UC ratio, cumulative	CD:UC ratio, b region of Ioannina	CD:UC ratio, region of Corfu	Perianal
Pre-1994	46.10%	1.4	30.4	0.23	0.26	0.2	13.80%
1995-1999	61.90%	3.4	35.4	0.32	0.46	0.3	27.20%
2000-2005	46.90%	1.55	33.1	0.61	> 1	0.5	31.30%
Total	51%	1.83	32.8	0.36	0.38	0.32	25.50%

was recorded in 23 (21.9%), fistulating in 25 (23.8%) and perianal disease in 26 (24.8%) CD patients. Of the UC patients, 71 (21.8%) had pancolitis, 99 (30.5%) had proctitis, and 155 (47.7%) had left-sided UC.

Mean annual crude incidence rates of CD and UC were 0.9 and 2.9/100 000 inhabitants, respectively. Age-adjusted incidence rates were 0.9/100 000 (95% CI 0.1-1.7) and 2.7 (95% CI 1.7-4.1), respectively. The age-adjusted mean annual incidence rate of IC was 0.2/100 000 (95% CI 0.1-0.4).

The incidence of CD was higher in men than in women. The highest incidence rate was found in the age group 18-44 years for men and women. UC incidence was higher in men also. The highest incidence rate was found in the age group 45-64 for both sexes.

There was a statistically significant increase of CD incidence ($P < 0.01$) during the study period, while the incidence of UC increased slightly and not in a statistically significant manner (Figure 1). As a consequence, the ratio of CD to UC practically doubled, surpassing 1 in the district of Ioannina, the largest population district and centre of the study, in the year 2002, and remaining above 1 for the following years (Table 2).

When demographic and clinical parameters were evaluated in correlation with the increase in CD incidence, age, gender, and regional patient distribution did not exhibit any statistically significant trends. There were also no differences in the percentages of patients with terminal ileal or colonic localization, and stenotic or fistulating disease during the pre-1995 period, compared to the 1995-1999 and the 2000-2005 periods. A statistically significant increase in the percentage of patients with perianal disease was observed in the latter two periods compared to the pre-1995 period (Table 2).

The association of CD with mutations in the three casual CARD15 variants is shown in Table 3.

There were no statistically significant differences on any mutations over time (Table 2).

Table 3 CARD15 variant allele frequencies

Samples	Genotype -/-	-/+	+/+	Mutant allele frequency (%)	*P*; OR (95% CI)
R702W					
CD	85	8	3	7.29	0.015; 3.85 (1.24-11.93)
UC	171	9	0	2.5	0.78; 1.26 (0.38-4.13)
Control	96	4	0	2	
G908R					
CD	83	13	0	6.77	0.70; 0.83 (0.39-1.78)
UC	156	24	0	6.66	0.61; 0.82 (0.42-1.58)
Control	84	16	0	8	
Leu1007fsincC					
CD	52	36	8	27.08	< 0.0001; 7.06 (3.46-14.37)
UC	166	9	5	5.27	1; 1.06 (0.48-2.32)
Control	90	10	0	5	

-/-, homozygous wild-type; -/+, heterozygous; +/+, homozygous mutant. CD: Crohn's disease; UC: Ulcerative colitis; OR: Odds Ratio; CI: Confidence Interval. The CARD15 gene variants determined in 96 CD patients (from 102 living CD patients out of 105, from our cohort 6 refused genetic test), 180 out of 300 living UC patients and 100 randomly chosen healthy blood donors.

DISCUSSION

North-western Greece is comprised of the mainland district of Epirus and the islands Corfu and Lefkada. Due to geographical reasons and relatively underdeveloped transport facilities to the rest of Greece the region can be considered secluded, and thus an ideal frame for epidemiological analysis of certain diseases. We have shown in the past that UC was a common entity in this region, while CD on the contrary was all too rare, with the CD:UC ratio in the 1980 s and early 1990 s being near 0.1[9,10]. Herein, the results of this 23-year period study, clearly show a rapid and sustained increase in CD incidence from 2000 on in North-western Greece, an increase that overturned the CD:UC ratio in the largest province of the study, the region of Ioannina, by 2002.

These findings are in accordance with formerly published studies showing an increasing trend for CD[16-22]. In these studies the increasing trend was often related to increasing age at diagnosis and could thus be related to improvements in average survival and surveillance (global increase[20,21] or a higher proportion of older patients[5,18,22]).

This is not the case in our study in which the majority of CD patients are young, similar to other studies[6,19] and the average patient age has not altered significantly over the years. Furthermore this increase could not be

attributed to enhanced surveillance and patient reporting; characteristically, the increase in incidence is predominantly expressed in the region of Ioannina, which, hosting the study centre for the past 25 years, guarantees total patient reporting and absence of bias. Moreover, a similar increase was not observed for UC during this period. This rising incidence suggests that unknown triggering factors continue to work in North-western Greece. To explain this increase in CD incidence throughout the period 1983-2005 we hypothesized that certain etiologic factors exist, and we evaluated the potential role of genetic susceptibility changing slowly over time in this incidence trend.

Although there are differences in ethnic, racial and geographic distribution[23], the genetic association of CD with CARD15 is undoubtedly replicated widely at present[24,25]. There were no significant differences in the prevalence of CARD15 variants during the study period in our study, indicating an absence of alterations in the genetic background. The prevalence of the three CARD15 variants is similar with that found in cohorts in central Europe and much higher than in northern Europe[25]. Yet these results are different from two other Greek studies: the first, based on the island of Crete outlined the rarity of SNP 13[26], an inconsistency that may be attributed to the fact that Crete is an isolated geographic region where this mutation does not seem to predispose to the disease, or to the relatively small size of the examined sample. The relatively higher frequencies in all three SNPs in the second study may be attributed to patient selection (hospitalized patients in a period of time, high proportion of ileocolonic disease)[27]. Our study, being population based, may be more representative of prevalence of CARD15 variants in Greece.

One could speculate that the increased incidence of CD recorded in recent years may actually reflect increase in detection, but not incidence of colonic disease, more easily recognized through increased endoscopy implementation. Although the total number of endoscopies performed naturally increased over time, there was no difference in endoscopy success rates, furthermore significant trends in disease localization over time were not recorded, apart from the already mentioned perianal CD (data not shown).

A potential criticism of the recruitment methods used in this study would be that ascertainment might have been incomplete, missing patients with CD or those with mild ulcerative proctitis who might have never required gastroenterologic consultation or hospital admission. However in the Greek population, during the time period of this study, there was an almost universal admission policy for investigation of IBD. Although North-western Greece has a relatively small population and isolated geographic area, this has actually been a great advantage and has resulted in a comprehensive study of the entire population, excluding any case leaks to other centers or existence of not notified cases followed-up by general practitioners.

In conclusion, we have shown that a significant increase in CD incidence in a secluded geographical region of Greece where CD was previously rare is not related to any alterations in the genetic profile or typical demographical factors. The study being population based outlines the need for further investigation of the interplay of environmental factors and disease.

ACKNOWLEDGMENTS

The authors thank the patients that participated in this study. The authors acknowledge the contribution of Dr M Gazouli for advice and technical support in genetic analysis, Kleopatra Garallea, Gioula Georgitsi, Lamprini Gouma, Alexandra Kioulou, for organization and collection of blood specimens and Drs E Zervou and M Tzilianos for helping to collect samples from healthy blood donors. We thank all gastroenterologists of North-western Greece Gastroenterology Group and Medical personnel of 1st Department of Internal Medicine University Hospital of Ioannina.

COMMENTS

Background

The incidence of Crohn's disease and ulcerative colitis varies greatly between specific geographic areas, and is supposed to be related to exogenous factors such as affluence and dietary habits, as well as endogenous factors such as genetic predisposition. North-western Greece was characterized by a low incidence of Crohn's disease (CD) although Ulcerative Colitis (UC) was not uncommon according to a previous study undertaken in the early 1990s.

Innovations and breakthroughs

The present study is one of the few to evaluate genetic predisposition to Crohn's disease and ulcerative colitis through time in a given area. The presence of absence of relation to incidence evolution allows for evaluation of the etiological role of genetic predisposition in disease.

Applications

Similar studies, when applied in larger areas, may be hampered by planning difficulties though.

Terminology

NOD2/CARD15 mutations are mutations of a specific genetic locus that have been shown to interfere with altered immune response and induce increased predisposition to the development of Crohn's disease.

Peer review

The author examined the relation between the change in the annual incidence rates of IBD patients and CARD15 variants in North-western Greece. Although the incidence of CD has significantly increased during the study period, there was no statisitical differences in NOD2/CARD15 variants among the Groups. The data is interesting and certainly provides the new evidences in this research field.

REFERENCES

1 **Podolsky DK**. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429
2 **Loftus EV**. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology* 2004; **126**: 1504-1517
3 **Shivananda S**, Lennard-Jones J, Logan R, Fear N, Price A, Carpenter L, van Blankenstein M. Incidence of inflammatory bowel disease across Europe: is there a difference between north and south? Results of the European Collaborative Study on Inflammatory Bowel Disease (EC-IBD). *Gut* 1996; **39**: 690-697
4 **Munkholm P**, Langholz E, Nielsen OH, Kreiner S, Binder V. Incidence and prevalence of Crohn's disease in the county of Copenhagen, 1962-87: a sixfold increase in incidence. *Scand J Gastroenterol* 1992; **27**: 609-614

5 **Lapidus A**, Bernell O, Hellers G, Persson PG, Löfberg R. Incidence of Crohn's disease in Stockholm County 1955-1989. *Gut* 1997; **41**: 480-486

6 **Molinié F**, Gower-Rousseau C, Yzet T, Merle V, Grandbastien B, Marti R, Lerebours E, Dupas JL, Colombel JF, Salomez JL, Cortot A. Opposite evolution in incidence of Crohn's disease and ulcerative colitis in Northern France (1988-1999). *Gut* 2004; **53**: 843-848

7 **Maté-Jimenez J**, Muñoz S, Vicent D, Pajares JM. Incidence and prevalence of ulcerative colitis and Crohn's disease in urban and rural areas of Spain from 1981 to 1988. *J Clin Gastroenterol* 1994; **18**: 27-31

8 **Trallori G**, Palli D, Saieva C, Bardazzi G, Bonanomi AG, d'Albasio G, Galli M, Vannozzi G, Milla M, Tarantino O, Renai F, Messori A, Amorosi A, Pacini F, Morettini A. A population-based study of inflammatory bowel disease in Florence over 15 years (1978-1992). *Scand J Gastroenterol* 1996; **31**: 892-899

9 **Tsianos EV**, Masalas CN, Merkouropoulos M, Dalekos GN, Logan RF. Incidence of inflammatory bowel disease in north west Greece: rarity of Crohn's disease in an area where ulcerative colitis is common. *Gut* 1994; **35**: 369-372

10 **Tsianos EV**, Katsanos KH, Christodoulou D, Dimoliatis I, Kogevinas A, Logan RF. Continuing low incidence of Crohn's disease in Northwest Greece. *Dig Liver Dis* 2003; **35**: 99-103

11 **Gasche C**, Scholmerich J, Brynskov J, D'Haens G, Hanauer SB, Irvine EJ, Jewell DP, Rachmilewitz D, Sachar DB, Sandborn WJ, Sutherland LR. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. *Inflamm Bowel Dis* 2000; **6:** 8-15

12 **Lennard-Jones JE**. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989; **170**: 2-6; discussion 16-19

13 **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

14 **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

15 **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

16 **Thomas GA**, Millar-Jones D, Rhodes J, Roberts GM, Williams GT, Mayberry JF. Incidence of Crohn's disease in Cardiff over 60 years: 1986-1990 an update. *Eur J Gastroenterol Hepatol* 1995; **7**: 401-405

17 **Moum B**, Vatn MH, Ekbom A, Aadland E, Fausa O, Lygren I, Stray N, Sauar J, Schulz T. Incidence of Crohn's disease in four counties in southeastern Norway, 1990-93. A prospective population-based study. The Inflammatory Bowel South-Eastern Norway (IBSEN) Study Group of Gastroenterologists. *Scand J Gastroenterol* 1996; **31**: 355-361

18 **Loftus EV**, Silverstein MD, Sandborn WJ, Tremaine WJ, Harmsen WS, Zinsmeister AR. Crohn's disease in Olmsted County, Minnesota, 1940-1993: incidence, prevalence, and survival. *Gastroenterology* 1998; **114**: 1161-1168

19 **Björnsson S**, Jóhannsson JH. Inflammatory bowel disease in Iceland, 1990-1994: a prospective, nationwide, epidemiological study. *Eur J Gastroenterol Hepatol* 2000; **12**: 31-38

20 **Lee FI**, Nguyen-van-Tam JS. Prospective study of incidence of Crohn's disease in northwest England: no increase since the late 1970's. *Eur J Gastroenterol Hepatol* 1994; **6**: 27-31

21 **Timmer A**, Breuer-Katschinski B, Goebell H. Time trends in the incidence and disease location of Crohn's disease 1980-1995: a prospective analysis in an urban population in Germany. *Inflamm Bowel Dis* 1999; **5**: 79-84

22 **Kyle J**. Crohn's disease in the northeastern and northern Isles of Scotland: an epidemiological review. *Gastroenterology* 1992; **103**: 392-399

23 **Economou M**, Trikalinos TA, Loizou KT, Tsianos EV, Ioannidis JP. Differential effects of NOD2 variants on Crohn's disease risk and phenotype in diverse populations: a metaanalysis. *Am J Gastroenterol* 2004; **99**: 2393-2404

24 **Vermeire S**, Rutgeerts P. Current status of genetics research in inflammatory bowel disease. *Genes Immun* 2005; **6**: 637-645

25 **Gaya DR**, Russell RK, Nimmo ER, Satsangi J. New genes in inflammatory bowel disease: lessons for complex diseases? *Lancet* 2006; **367**: 1271-1284

26 **Roussomoustakaki M**, Koutroubakis I, Vardas EM, Dimoulios P, Kouroumalis EA, Baritaki S, Koutsoudakis G, Krambovitis E. NOD2 insertion mutation in a Cretan Crohn's disease population. *Gastroenterology* 2003; **124**: 272-273; author reply 273-274

27 **Gazouli M**, Zacharatos P, Mantzaris GJ, Barbatis C, Ikonomopoulos I, Archimandritis AJ, Lukas JC, Papalambros E, Gorgoulis V. Association of NOD2/CARD15 variants with Crohn's disease in a Greek population. *Eur J Gastroenterol Hepatol* 2004; **16**: 1177-1182

S- Editor Zhu LH **L- Editor** Rampone B **E- Editor** Liu Y

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5121-5126
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Lead exposure increases oxidative stress in the gastric mucosa of HCl/ethanol-exposed rats

Samuel Babafemi Olaleye, Oluwatosin Adekunle Adaramoye, Perebiri Peter Erigbali, Olasupo Sunday Adeniyi

Samuel Babafemi Olaleye, Perebiri Peter Erigbali, Olasupo Sunday Adeniyi, Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria
Oluwatosin Adekunle Adaramoye, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria
Supported by the Senate, University of Ibadan, Nigeria partly through SRG grant to SBO UI/SRG/COM/2000/10A
Correspondence to: Samuel Babafemi Olaleye, PhD, Gastrointestinal Research Unit, Department of Physiology, University of Ibadan, Ibadan, Nigeria. sb.olaleye@mail.ui.edu.ng
Telephone: +234-802-3255893 Fax: +234-802-28711135
Received: March 1, 2007 Revised: July 6, 2007

Abstract

AIM: To investigate the role of reactive oxygen species in the ulcer-aggravating effect of lead in albino rats.

METHODS: Albino Wistar rats were randomly divided into three groups and treated orally with 100 mg/L (low dose) or 5000 mg/L (high dose) of lead acetate for 15 wk. A third group received saline and served as control. At the end of wk 15, colorimetric assays were applied to determine the concentrations of total protein and nitrite, the activities of the oxidative enzymes catalase and superoxide dismutase, and lipid peroxidation in homogenized gastric mucosal samples.

RESULTS: Exposure of rats to lead significantly increased the gastric mucosal damage caused by acidified ethanol. Although the basal gastric acid secretory rate was not significantly altered, the maximal response of the stomach to histamine was significantly higher in the lead-exposed animals than in the unexposed control group. Exposure to low and high levels of lead significantly increased gastric lipid peroxidation to 183.2% ± 12.7% and 226.1% ± 6.8% of control values respectively ($P < 0.0$). On the other hand, lead exposure significantly decreased catalase and superoxide dismutase (SOD) activities and the amount of nitrite in gastric mucosal samples.

CONCLUSION: Lead increases the formation of gastric ulcers by interfering with the oxidative metabolism in the stomach.

Key words: Lead; Ulcer; Lipid peroxidation; Catalase; Gastric acid

Olaleye SB, Adaramoye OA, Erigbali PP, Adeniyi OS. Lead exposure increases oxidative stress in the gastric mucosa of HCl/ethanol-exposed rats. *World J Gastroenterol* 2007; 13(38): 5121-5126

http://www.wjgnet.com/1007-9327/13/5121.asp

INTRODUCTION

Reactive oxygen species (ROS) have been shown to be to be involved in the etiology of many inflammatory disorders of the gastrointestinal system[1,2]. This is evidenced by the increased oxidative stress by pro-ulcerative factors in the gut such as *H pylori*[3,4], use of non-steroidal anti-inflammatory drugs[5], smoking[6], psychological stress, corticosteroid use[7], and loss of sleep[8]. Lipid peroxidation (LPO), a result of the reaction of oxyradicals and polyunsaturated acids, has been suggested as an attack factor in the gastric mucosa[9]. Also GSH, an endogenous sulfhydryl compound, is an important substance in the cellular defense system[10].

Nitric oxide (NO) depletion also plays significant roles in the pathogenesis of gastric ulcers. NO along with superoxide ($0_2^{\cdot}$) and the products of their interaction initiate a wide range of toxic oxidative reactions causing tissue injury[11]. Large amounts of NO, generated primarily by iNOS, can be toxic and pro-inflammatory. Likewise, neutrophils too produce oxidants and release granular constituents comprising lytic enzymes performing an important role in inflammatory injury[12].

Although lead acetate does not initiate any excess generation of reactive oxygen species in a cerebral synaptosomal suspension, it has a marked ability to enhance the pro-oxidant properties of ferrous iron in the same system[13]. It also amplifies oxidative stress induced by l-glutamate[14]. Recent studies suggest that accumulated lead exposure is related to several chronic disorders of aging including disorders that have been associated with oxidative stress[15-17]. Sass[19] first reported that lead poisoning in dogs is associated with perforating gastrointestinal ulcers. Bercowitz and Laufer[19] observed that gastrointestinal ulcer patients had accumulated lead in their teeth, thus suggesting a relationship between lead and ulcer. In a previous study, we reported that long term exposure of rats to lead predisposes the stomach to higher ulcerogenic effects of indomethacin and acidified ethanol[20]. However, the exact mechanism(s) by which lead

promotes gastric ulceration is not yet understood. The objective of the present study thus was to investigate the involvement of ROS in the aggravation of ulceration in the stomach of lead-exposed rats.

MATERIALS AND METHODS

Chemicals and animals

Lead acetate and urethane were obtained from BDH chemicals Ltd, Poole. England. Epinephrine, 5,5-dithio-bis-2-nitrobenzoic acid, hydrogen peroxide, sodium pentobarbitone, and thiobarbituric acid (TBA) were purchased from Sigma (St Loius, MO USA). All other reagents were of analytical grade and were obtained from the British Drug Houses, Poole, UK.

Young male albino Wistar rats (80-90 g) were obtained from the small animal house, College of Medicine, University of Ibadan, Nigeria. They were randomly divided into three groups with adequate matching of weight. They were kept in wire meshed cages and fed with commercial rat pellets (Ladokun Feeds Ltd, Ibadan, Nigeria) and allowed to take water ad libitum.

Lead treatment

Animals were exposed to lead as previously described[20]. The high lead group (HiPb) was given 5000 mg/L lead acetate in drinking water daily while the low lead group (LoPb) received 100 mg/L lead acetate in drinking water. The control animals received only drinking water. At the end of a 15 wk exposure, blood samples were collected from the rats for analysis of the Packed Cell Volume (PCV), red and white blood cell counts, and neutrophile numbers by standard methods. Afterwards, ulcer was induced as follows.

Induction of experimental ulcer

Acidified ethanol was used to induce experimental gastric ulcers. Thirty six hours fasted rats were given 1.0 mL of HCl/ethanol mixture containing 0.15 mol/L HCl in 70% mL/L ethanol[21]. Four hours after administering the ulcerogen and under sodium pentobarbitone anaesthesia (60 mg/kg, ip), the animals were sacrificed. The stomachs were removed, inflated with 10 mL of 2% formaldehyde for 10 min to fix the tissue walls and opened along the greater curvature. The hemorrhagic lesions were stretched out on a glass plate and their sizes were estimated using an underlying graph paper with a 1 mm^2 grid. Lesion areas were summed up per stomach and expressed as % of total mucosal area.

Measurement of gastric acid secretion

Gastric acid secretion was studied in the 3 groups using a modification of the method originally described by Ghosh[22]. The animals were fasted for 24 h at the end of the 15 wk lead treatment. Under urethane anaesthesia [250 g/L urethane (6 mL/kg body, ip)], the animals were surgically prepared for in situ stomach perfusion. The pylorus was semi-transected at its junction with the duodenum and a pyloric cannula inserted and tied into place. An oesophageal cannula for infusion from a flow meter (Watson-Marlow Inc., USA) was passed through the mouth to the stomach. Ten minute effluent samples (10.0 ± 0.1 mL each) were collected *via* a pyloric cannula and titrated against 0.01 mol/L NaOH using phenolphthalein as indicator. Acidity was expressed in mol/L/L per min life. After obtaining a consistent basal acid output, each animal was injected *via* a femoral vein cannula with either 9 g/L NaCl or histamine acid phosphate.

Biochemical analysis

At the end of chronic lead exposure, the animals were killed under deep ether anaesthesia and the stomachs were removed. Each stomach was cut open through the greater curvature, rinsed in normal saline, weighed, and homogenized in 10 mL KCl (pH = 7.4). The homogenate was subsequently used for total protein estimation. The protein content of the gastric mucosa was estimated by the method of Lowry *et al*[23] using bovine serum albumin as a standard.

Determination of lipid peroxidation. Lipid peroxidation was assayed by measuring the thiobarbituric acid reactive (TBAR) products using the procedure of Walls *et al*[24]. Briefly, the homogenate was supplemented with 0.75 g/L TBA in 0.1 mol/L HCl. The reactants were then supplemented with 5 mL n-butanol-pyridine mixture, shaken vigorously for 1 min and centrifuged for 10 min at 4000 r/min. Absorbance was then read at 532 nm and the results expressed as nmol TBA per 100 mg wet tissue.

Determination of catalase activity. Activity of catalase in gastric mucosa was determined according to the procedure of Sinha[25]. This method is based on the reduction of dichromate in acetic acid to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate so produced is measured calorimetrically at 530 nm.

Determination of SOD activity. For determination of SOD activity, samples of gastric mucosal homogenates were taken as described above. A method originally described by Misra and Fridovich[26] as reported by Magwere *et al*[27] was employed. This method is based on the ability of superoxide dismutase to inhibit the autoxidation of epinephrine caused by O_2 generated by xanthine oxidase reaction.

Determination of nitrite in gastric mucosa. The gastric mucosa was cooled in ice-cold distilled water before homogenization (100 g/L). The crude homogenate was centrifuged at 21.000 g for 20 min at 4℃. Aliquots of the supernatants were taken to determine nitrite levels. The amounts of nitrite were measured in the gastric mucosa by performing the Griess reaction. 100 mL of sample were incubated with 100 mL of Griess reagent (Sigma) at room temperature for 20 min. Nitrite level was determined by measuring the absorbance at 550 nm using a spectrophotometer (DU 640B, Beckman, Fullerton, California, USA)[28,29].

Determination of gastric mucous. Adherent gastric glandular mucous was measured by the method of Corne *et al*[30]. Excised gastric glandular portion of the stomachs were transferred for 2 h to 0.1% Alcian blue dissolved

Table 1 Effects of lead exposure on acidified ethanol-induced gastric injury (mean ± SEM, *n* = 8)

Treatment	Mass gain (% of initial mass)	Mucosal damage score (% of total mucosal area)	Total protein (mg/g)	Gastric mucus (mg/g)
Control	43.3 ± 4.4	6.94 ± 0.42	9.80 ± 0.01	31.0 ± 0.31
Low lead	17.4 ± 6.3[a]	9.72 ± 0.65[a]	7.50 ± 0.14	28.0 ± 0.27[a]
High lead	6.3 ± 5.1[a]	12.50 ± 0.51[a]	6.20 ± 0.01	23.0 ± 0.24[a]

[a]$P < 0.05$ *vs* control.

in buffer solution containing 0.1 mol/L sucrose and 0.05 mol/L sodium acetate (pH adjusted to 5.8 with hydrochloric acid). After washing the stomach twice in 0.25 mol/L sucrose (15 min and 45 min), the dye complexed with mucous was eluted by immersion in 10 mL aliquots of 0.5 mol/L $MgCl_2$ for 2 h. The resulting blue solution was shaken with equal volumes of diethyl ether and the optical density of the aqueous phase was measured spectrophotometerically at 605 nm.

Statistical analysis

All values presented in tables are expressed as mean ± SEM. The appropriate comparisons between groups were made using Student' *t*-test. The difference between the groups is taken to be significant at $P < 0.05$.

RESULTS

Development of gastric lesions

Administration of acidified ethanol caused severe damage to the rat stomachs under investigation. The site of the formation of lesions is the corpus mucosa. The mean ulcer score in the control animals was 6.94% ± 0.42%. The severity of lesions was markedly increased by chronic exposure to both low and high lead concentrations (Table 1, $P < 0.05$). Also shown in Table 1 is the result of the total protein and adherent mucous content of the glandular stomach. Long term exposure to lead significantly decreased both gastric mucous and protein contents, the inhibition being greatest in the high lead treated group.

Basal rate of gastric acid secretion was not significantly affected by lead exposure. However, the maximal response of the stomach of lead-exposed animals to histamine was significantly higher than those of the unexposed control group (Figure 1).

Lipid peroxidation and gastric antioxidant enzymes

Lipid peroxidation (measured as the amount of TBA reactants in the gastric mucosa) in the unexposed control animals was 595 ± 82 mmol/g tissue. Exposure of rats to low and high lead levels significantly increased gastric TBA reactant level to 183.2% ± 12.7% and 226.1% ± 6.8% of the control value, respectively (Figure 2A). On the other hand, SOD activity in the intact gastric mucosa (control group) averaged 334 ± 22 nkat/g. Exposure of rats to low and high lead levels significantly decreased SOD activity to 78% ± 3% and 66% ± 6% of the control, respectively

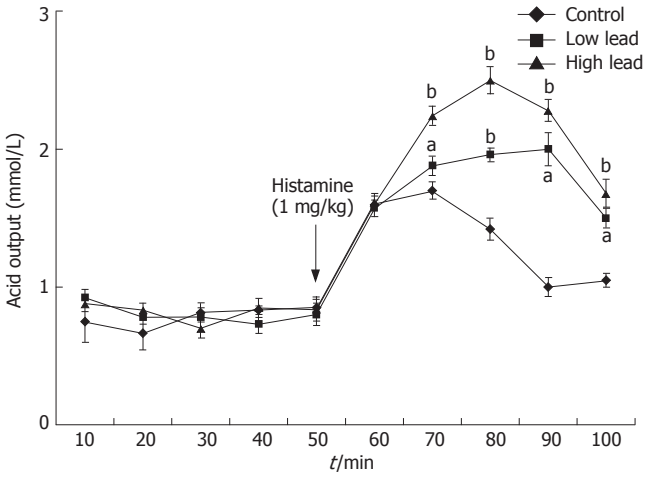


Figure 1 Gastric acid secretory output after administration of histamine (1 mg/kg) in rats ([a]$P < 0.05$, [b]$P < 0.01$ *vs* control).

(Figure 2B). Similarly, lead exposure decreases gastric mucosal catalase activity to 88.22% ± 3.21% and 55.29% ± 2.15% of the control, respectively (Figure 2C).

Gastric mucosal nitrite concentration

The control gastric mucosal nitrite level was 62.4 ± 4.2 nmol/g tissue (Figure 3). Chronic exposure of rats to lead for 15 wk depleted the gastric nitrite level to 51.6 ± 3.1 nmol/g tissue ($P < 0.05$) and 49.7 ± 2.5 nmol/g tissue ($P < 0.01$).

DISCUSSION

The present study investigated the role of oxidative stress in the ulcer-promoting action of prolonged lead exposure in rats. The doses and the duration of lead treatment used in our study have been shown to cause significant high blood lead levels in previous studies[20,31]. Thus, there is no doubt that the animals had high blood lead levels. The acidified ethanol model has been used widely to produce gastric mucosal damage[21]. We observed that long-term exposure of rats to lead given through drinking water significantly increased the incidence of gastric ulcer produced by the ulcerogen. This agrees with our previous report in which lead was shown to aggravate gastric ulcers induced by indomethacin and acidified ethanol[20].

It is well established that gastric acid secretion plays a role in gastric ulcer. Moreover, many anti-ulcerogenic drugs act by reducing the acid secretion[32]. The present result suggests that basal gastric acid secretory rate was not significantly affected by prolonged lead treatment. However, maximal stimulation by the established secretagogue, histamine, was markedly increased in the lead exposed rats. We had earlier reported that acid output from pylorus ligated stomach of lead exposed rats was increased by lead treatment. Further studies may therefore be required using different acid secretory models before a conclusion can be made on the exact effect of lead exposure on gastric acid secretion.

Lipid peroxidation has been implicated in the etiology

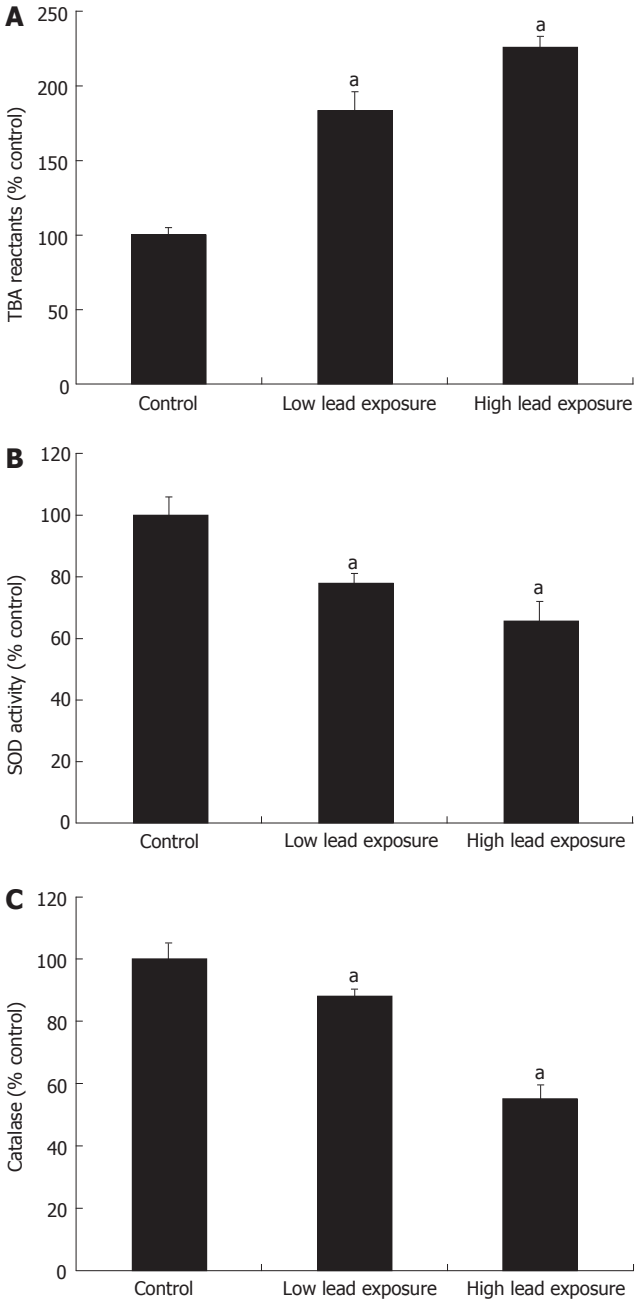


Figure 2 TBA reactants, superoxide dismutase and catalase activities in gastric mucosa of rats exposed to chronic lead treatments (mean ± SEM, $n = 8$, $^{a}P < 0.05$ *vs* control).

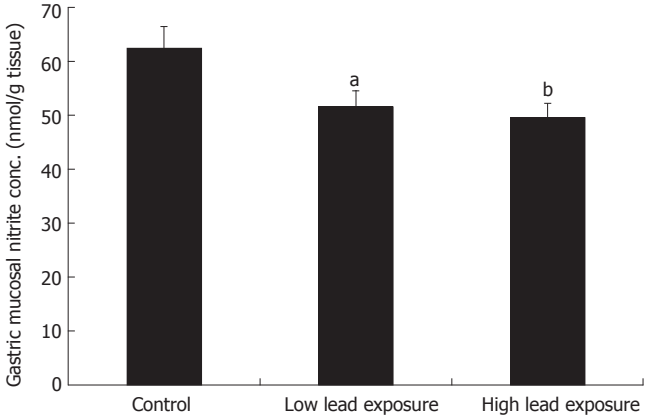


Figure 3 Gastric mucosal nitrite concentration in lead exposed rats (mean ± SEM, $n = 8$ rats, $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control).

of damage to subcellular membranes and then injury in the cell. In the present study, lipid peroxidation, as measured by the amount of TBA reactants, was increased by lead exposure. The implication of this is that lead causes an increase in the formation of free radicals, which, if not mopped up by free radical scavengers as SOD, CAT, or glutathione, will expose the stomach to inflammation.

Wapnir *et al*[33] reported that juvenile rats fed a diet containing 1% lead acetate for 7 wk suffered from malabsorption of certain amino acids, as the intestinal absorption of glycine, lycine, and phenylalanine were decreased. Furthermore, administration of HCl and ethanol has been shown to produce ulcerative lesions and increased lipid peroxidation in the gastric mucosa with depletion of endogenous antioxidants[21]. The result of this study showed a significant decrease in the protein glandular protein level of the rats given low and high doses of lead acetate. This may also explain the significant low levels of the antioxidizing enzymes SOD and CAT observed in the treated groups.

In gastroprotection, the first line of antioxidative enzyme is SOD which catalyses the dismutation of superoxide radical anion (O_2) into less noxious hydrogen peroxide (H_2O_2). H_2O_2 is then inactivated by the degradation into water by catalase or glutathione peroxidase[35,36]. Depletion of these enzymes, as evident by the results of this study, therefore predisposes the stomach to a greater impact of the free radicals produced *via* increased lipid peroxidation, hence increased ulcer formation following lead exposure.

Apart from the free radical scavenging enzymes, another agent that has been widely believed to be involved in the regulation of various gastric functions and in the modulation of gastric mucosal integrity is nitric oxide (NO). This is evidenced by reports showing that suppression of NO production by NO synthase inhibitor worsens gastric lesions induced by ethanol in rats[37,38]. NO has also been suggested to prevent infiltration by neutrophils, which are a source of superoxide radical anions[39]. The reduction in the gastric tissue nitrite in the lead treated groups in the present study is therefore indicative for a reduced protective capacity of endogenous NO.

In summary, chronic exposure of rats to lead intensifies HCl/ethanol-induced gastric mucosal damage and this may be related to reduced gastric contents of endogenous NO and the antioxidative enzymes SOD and CAT.

COMMENTS

Background

A number of humans are occupationally exposed to high levels of lead through paints, car exhausts battery fumes, *etc*. Lead exposure also occurs through ingestion with food and via inhalation. Previous studies in dogs and rats have shown that long-term exposure to high level lead in drinking water predisposes the stomach to ulcer. The mechanism of this effect of lead is not understood.

Research frontiers

Oxidative stress has been shown to be a major causative factor for many diseases, including gastrointestinal ulcers. The hotspot of this study is to examine if the increased susceptibility of the stomach to ulcer after prolonged lead exposure can be explained (partly or totally) by an increased oxidative stress in the stomach.

Innovations and breakthroughs

The results of this study show that chronic exposure of rats to lead intensifies HCl/ethanol-induced gastric mucosal damage and this may be related to reduced gastric contents of endogenous nitric oxide and antioxidative enzymes.

Applications

The study suggests that prolonged exposure to lead may be a factor in the etiology of gastrointestinal ulcers.

Terminology

Oxidative stress: a condition of increased oxidant production in animal cells characterized by the release of free radicals and resulting in cellular degeneration. Free radicals damage components of the cells' membranes, proteins, or genetic material by "oxidizing" them-the same chemical reaction that causes iron to rust; Lead: a soft heavy toxic malleable metallic element; bluish white when freshly cut but tarnishes readily to dull grey; Lead poisoning: Lead can come into the body in a number of ways: through water that goes through lead pipes, through badly canned food and through small pieces of paint. Victims of lead poisoning may get headaches, dizziness, confusion, and problems seeing. They may also become slowly paralyzed, starting with the hands. In very serious cases, it can cause death.

Peer review

This is an interesting and generally well written paper with some new information.

REFERENCES

1 **Perry MA**, Wadhwa S, Parks DA, Pickard W, Granger DN. Role of oxygen radicals in ischemia-induced lesions in the cat stomach. *Gastroenterology* 1986; **90**: 362-367
2 **Schmassmann A**, Stettler C, Poulsom R, Tarasova N, Hirschi C, Flogerzi B, Matsumoto K, Nakamura T, Halter F. Roles of hepatocyte growth factor and its receptor Met during gastric ulcer healing in rats. *Gastroenterology* 1997; **113**: 1858-1872
3 **Yamaguchi N**, Kakizoe T. Synergistic interaction between Helicobacter pylori gastritis and diet in gastric cancer. *Lancet Oncol* 2001; **2**: 88-94
4 **Janulaityte-Günther D**, Günther T, Pavilonis A, Kupcinskas L. What Bizzozero never could imagine - Helicobacter pylori today and tomorrow. *Medicina* (Kaunas) 2003; **39**: 542-549
5 **Rostom A**, Wells G, Tugwell P, Welch V, Dube C, McGowan J. Prevention of chronic NSAID induced upper gastrointestinal toxicity. *Cochrane Database Syst Rev* 2000; CD002296
6 **Ma L**, Wang WP, Chow JY, Yuen ST, Cho CH. Reduction of EGF is associated with the delay of ulcer healing by cigarette smoking. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G10-G17
7 **Levenstein S**. Peptic ulcer at the end of the 20th century: biological and psychological risk factors. *Can J Gastroenterol* 1999; **13**: 753-759
8 **Bercovitz K**, Laufer D. Lead accumulation in teeth of patients suffering from gastrointestinal ulcers. *Sci Total Environ* 1991; **101**: 229-234
9 **Guo JS**, Chau JF, Cho CH, Koo MW. Partial sleep deprivation compromises gastric mucosal integrity in rats. *Life Sci* 2005; **77**: 220-229
10 **Hung CR**. Importance of histamine, glutathione and oxyradicals in modulating gastric haemorrhagic ulcer in septic rats. *Clin Exp Pharmacol Physiol* 2000; **27**: 306-312
11 **Poli G**, Albano E, Dianzani MU. Free radicals: From Basic Science to Medicine. Basel: Birkhauser Verlag AG, 1993: 18-30
12 **Hogg N**. Free radicals in disease. *Semin Reprod Endocrinol* 1998; **16**: 241-248
13 **Yoshikawa T**, Naito Y. The role of neutrophils and inflammation in gastric mucosal injury. *Free Radic Res* 2000; **33**: 785-794
14 **Bondy SC**, Guo SX. Lead potentiates iron-induced formation of reactive oxygen species. *Toxicol Lett* 1996; **87**: 109-112
15 **Naarala JT**, Loikkanen JJ, Ruotsalainen MH, Savolainen KM. Lead amplifies glutamate-induced oxidative stress. *Free Radic Biol Med* 1995; **19**: 689-693
16 **Romero-Alvira D**, Roche E. High blood pressure, oxygen radicals and antioxidants: etiological relationships. *Med Hypotheses* 1996; **46**: 414-420
17 **Thylefors B**, Négrel AD, Pararajasegaram R, Dadzie KY. Available data on blindness (update 1994) *Ophthalmic Epidemiol* 1995; **2**: 5-39
18 **Mecocci P**, Mariani E, Cornacchiola V, Polidori MC. Antioxidants for the treatment of mild cognitive impairment. *Neurol Res* 2004; **26**: 598-602
19 **Sass B**. Perforating gastric ulcer associated with lead poisoning in a dog. *J Am Vet Med Assoc* 1970; **157**: 76-78
20 **Olaleye SB**, Raji Y, Onasanwo SA, Erigbali P, Oyesola SO, Odukanmi A, Omotosho IO, Elegbe RA. Potentiation of Gastric Ulceration By Experimental Lead Exposure In Rats. *J Biol Sci* 2006; **6**: 480-484
21 **Anadan R**, Rekha RD, Saravanan N, Devaki T. Protective effects of Picrorrhiza kurroa against HCl/ethanol induced ulceration in rats. *Fitoterapia* 1999; **70**: 498-503
22 **Ghosh MN**, Schild HO. Continuous recording of acid gastric secretion in the rat. *Br J Pharmacol Chemother* 1958; **13**: 54-61
23 **Lowry OH**, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265-275
24 **Walls R**, Kumar KS, Hochstein P. Aging human erythrocytes. Differential sensitivity of young and old erythrocytes to hemolysis induced by peroxide in the presence of thyroxine. *Arch Biochem Biophys* 1976; **174**: 463-468
25 **Sinha AK**. Colorimetric assay of catalase. *Anal Biochem* 1972; **47**: 389-394
26 **Misra HP**, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972; **247**: 3170-3175
27 **Magwere T**, Naik YS, Hasler JA. Effects of chloroquine treatment on antioxidant enzymes in rat liver and kidney. *Free Radic Biol Med* 1997; **22**: 321-327
28 **Green LC**, Ruiz de Luzuriaga K, Wagner DA, Rand W, Istfan N, Young VR, Tannenbaum SR. Nitrate biosynthesis in man. *Proc Natl Acad Sci USA* 1981; **78**: 7764-7768
29 **Crespo E**, Macías M, Pozo D, Escames G, Martín M, Vives F, Guerrero JM, Acuña-Castroviejo D. Melatonin inhibits expression of the inducible NO synthase II in liver and lung and prevents endotoxemia in lipopolysaccharide-induced multiple organ dysfunction syndrome in rats. *FASEB J* 1999; **13**: 1537-1546
30 **Corne SJ**, Morrissey SM, Woods RJ. Proceedings: A method for the quantitative estimation of gastric barrier mucus. *J Physiol* 1974; **242**: 116P-117P
31 **Gruber HE**, Gonick HC, Khalil-Manesh F, Sanchez TV, Motsinger S, Meyer M, Sharp CF. Osteopenia induced by long-term, low- and high-level exposure of the adult rat to lead. *Miner Electrolyte Metab* 1997; **23**: 65-73
32 **Schmassmann A**. Mechanisms of ulcer healing and effects of nonsteroidal anti-inflammatory drugs. *Am J Med* 1998; **104**: 43S-51S; discussion 79S-80S
33 **Wapnir RA**, Exeni RA, McVicar M, Lipshitz F. Experimental lead poisoning and intestinal transport of glucose, amino acids, and sodium. *Pediatr Res* 1977; **11**: 153-157
34 **Blum J,** Fridovich I. Inactivation of glutathione peroxidase by superoxide radical. *Arch Biochem Biophys* 1985; **240**: 500-508
35 **Kwiecień S**, Brzozowski T, Konturek PC, Pawlik MW, Pawlik WW, Kwiecień N, Konturek SJ. Gastroprotection by pentoxyfilline against stress-induced gastric damage. Role of lipid peroxidation, antioxidizing enzymes and proinflammatory cytokines. *J Physiol Pharmacol* 2004; **55**: 337-355

36 **Masuda E**, Kawano S, Nagano K, Tsuji S, Takei Y, Tsujii M, Oshita M, Michida T, Kobayashi I, Nakama A. Endogenous nitric oxide modulates ethanol-induced gastric mucosal injury in rats. *Gastroenterology* 1995; **108**: 58-64
37 **Konturek SK**, Konturek PC. Role of nitric oxide in the digestive system. *Digestion* 1995; **56**: 1-13
38 **Gaboury J**, Woodman RC, Granger DN, Reinhardt P, Kubes P. Nitric oxide prevents leukocyte adherence: role of superoxide. *Am J Physiol* 1993; **265**: H862-H867
39 **Okcu N**, Onuk MD, Yilmaz A, Gundogdu M, Baran T. The effects of omeprazole and ranitidine on the gastric ulcer healing. *Doga Trop J Med Sci* 1992; **16**: 657-658

S- Editor Ma N **L- Editor** Mihm S **E- Editor** Lu W

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5127-5132
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

N-acetylcysteine attenuates oxidative stress and liver pathology in rats with non-alcoholic steatohepatitis

Duangporn Thong-Ngam, Suchittra Samuhasaneeto, Onanong Kulaputana, Naruemon Klaikeaw

Duangporn Thong-Ngam, Suchittra Samuhasaneeto, Onanong Kulaputana, Naruemon Klaikeaw, Department of Physiology; Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
Supported by Grant of Ratchadapisek Somphok, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand
Correspondence to: Duangporn Thong-Ngam, MD, Department of Physiology, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand. thongngam007@yahoo.com
Telephone: +66-2-2564267 Fax: +66-2-2564267
Received: May 17, 2007 Revised: August 3, 2007

Abstract

AIM: To evaluate attenuating properties of N-acetylcysteine (NAC) on oxidative stress and liver pathology in rats with non-alcoholic steatohepatitis (NASH).

METHODS: Male Sprague-Dawley rats were randomly divided into three groups. Group 1 (control, *n* = 8) was free accessed to regular dry rat chow (RC) for 6 wk. Group 2 (NASH, *n* = 8) was fed with 100% fat diet for 6 wk. Group 3 (NASH + NAC_{20}, *n* = 9) was fed with 100% fat diet plus 20 mg/kg per day of NAC orally for 6 wk. All rats were sacrificed to collect blood and liver samples at the end of the study.

RESULTS: The levels of total glutathione (GSH) and hepatic malondialdehyde (MDA) were increased significantly in the NASH group as compared with the control group (GSH; 2066.7 ± 93.2 *vs* 1337.5 ± 31.5 μmol/L and MDA; 209.9± 43.9 *vs* 3.8 ±1.7 μmol/g protein, respectively, $P < 0.05$). Liver histopathology from group 2 showed moderate to severe macrovesicular steatosis, hepatocyte ballooning, and necroinflammation. NAC treatment improved the level of GSH (1394.8 ± 81.2 μmol/L, $P < 0.05$), it did not affect MDA (150.1 ± 27.0 μmol/g protein), but led to a decrease in fat deposition and necroinflammation.

CONCLUSION: NAC treatment could attenuate oxidative stress and improve liver histology in rats with NASH.

Key words: N-acetylcysteine; Oxidative stress; Non-alcoholic steatohepatitis

Thong-Ngam D, Samuhasaneeto S, Kulaputana O, Klaikeaw N. N-acetylcysteine attenuates oxidative stress and liver pathology in rats with non-alcoholic steatohepatitis. *World J Gastroenterol* 2007; 13(38): 5127-5132

http://www.wjgnet.com/1007-9327/13/5127.asp

INTRODUCTION

Non-alcoholic steatohepatitis (NASH) is a liver disease characterized by macrovesicular steatosis, hepatocyte necrosis, inflammation, Mallory bodies, and fibrosis[1]. NASH is closely associated with the metabolic or insulin resistance syndrome[2]. This is a cluster of disorders, such as obesity, diabetes mellitus, dyslipidemia, arteriosclerosis, and hypertension, with insulin resistance as a common feature[3]. In initial phases, during which fat accumulates in the liver, no clinical symptoms are evident. In advanced stages, fibrosis is detectable, which might progress into cirrhosis in some patients[4].

There are many models of NASH-like liver injuries in animals as the genetic model of *ob/ob* mice[5], the methionine and choline deficient diet model[6,7], and a model with high-fat liquid diet in which 71% of energy is derived from fat, 11% from carbohydrates, and 18% from protein[8].

Oxidative stress is believed to play an important role in pathogenesis of NASH. It is likely involved in the progression of disease from steatosis to NASH and potentially cirrhosis. It has been shown that chronic oxidative stress, generated through the oxidation of cytotoxic free fatty acids, can lead to upregulation of cytokines[9], induction of the liver cytochrome P450 enzyme 2E1 (CYP2E1), and depletion of hepatic antioxidant concentration[6]. In addition, enhanced lipid peroxidation leads to the generation of byproducts, such as 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA), which have been shown to further stimulate cytokine production. They are involved in hepatic stellate cell activation[10], fibrogenesis, and enhanced extracellular matrix protein deposition.

According to the concepts of pathogenesis of NASH, these might make a wise basis for the use of antioxidants or drugs that could protect hepatocytes from oxidative stress. N-acetylcysteine (NAC) is a glutathione precursor which increases glutathione levels in hepatocytes[11]. Increased glutathione levels, in turn, limit the production of reactive oxygen species (ROS)

which cause hepatocellular injury[12]. Oral NAC treatment (1 g/d) of 11 NASH patients for 3 mo was demonstrated to improve liver function test significantly at the end of treatment period[11]. In a controlled study, NAC (600 mg/d) was administered to NASH patients for 4 wk, and a significant improvement in aminotransferase levels was found[13]. Although NAC was shown to improve liver function test in NASH patients, the mechanism remained unclear. Treatment of NASH with diet or diet plus NAC could attenuate oxidative stress as well as improve biochemical parameters and liver histopathology. However, the result of addition of NAC is not better than diet treatment alone[14]. Therefore, this study was conducted to determine the effects of NAC on oxidative stress and liver pathology in a rat model of 100% fat diet induced NASH[15].

MATERIALS AND METHODS

Animal preparation

This study was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand. Male Sprague-Dawley rats weighing 220-260 g from the National Laboratory Animal Center, Mahidol University, Salaya, Nakorn Pathom were used. The animals were allowed to rest for a week after arrival at the Animal Center, Department of Physiology, Faculty of Medicine, Chulalongkorn University. They were kept at a controlled temperature of 25 ± 1℃ under standard conditions (12 h dark: 12 h light cycle), fed with regular dry rat chow ad libitum, and had freely access to drinking water.

Experimental protocols

Rats were randomly divided into three experimental groups. Group 1: Fed ad libitum with regular dry rat chow for 6 wk (control group, n = 8). Group 2: Fed ad libitum with 100% fat diet for 6 wk to induce NASH (NASH group, n = 8). Group 3: Fed ad libitum with 100% fat diet plus 20 mg/kg per day of NAC orally (NASH + NAC_{20} group, n = 9) for 6 wk.

All rats were weighed weekly. They were sacrificed to collect blood, serum, and liver samples at the end of the study, 20 h after the last NAC treatment. The diagram of the experiment was shown as follow.

At the end of the study, all rats were anaesthetized using intraperitoneal injection of an overdose (45 mg/kg) of sodium pentobarbital, and the abdominal walls were opened. Blood was drawn by cardiac puncture for total glutathione assay and biochemical assay. The livers were excised quickly and cleaned in iced-cold NSS. One lobe of the liver was collected for MDA measurement, the remaining liver was fixed in 40 g/L formaldehyde solution for histological examination.

Total glutathione determination

Total glutathione levels were quantified using Cayman's GSH assay kit. This assay uses glutathione reductase for determination of glutathion. The sulfhydryl group of glutathion reacts with DTNB (5, 5'-dithiobis-2-nitrobenzoic acid, Ellman's reagent) and produces a yellow colored 5-thio-2-nitrobenzoic acid (TNB). The mixed disulfide, GSTNB (between glutathion and TNB) that is concomitantly produced, is reduced by glutathione reductase to recycle glutathion and to produce more TNB. The rate of TNB production is directly proportional to this recycling reaction which is in turn directly proportional to the concentration of glutathionn in the sample. Measurement of the absorbance of TNB at 405 nm provides an accurate estimation of glutathion in the sample.

Hepatic malondialdehyde (MDA) determination

One lobe of the liver was removed and weighed. One gram of the tissue was placed in a test tube containing 2.25 mL homogenization buffer (11.5 g/L KCl) and homogenized in an ice box using a homogenizer at a rotational speed of 12000 r/min for 1 min. MDA was quantified by using the thiobarbituric acid reaction as described by Ohgawa *et al*[16]. MDA levels in the samples were determined the linear regression equation from a standard curve. The content of lipid peroxide is expressed as nmol of MDA/g of wet weight, and the total protein was determined by the Lowry method[17] to correct the MDA level which is expressed in terms of μmol/g protein.

Histopathological examination

The remaining liver samples were fixed in 40 g/L formaldehyde solution at room temperature. They were processed by standard methods. Briefly, tissues were embedded in paraffin, sectioned at 5 μm, stained with HE, and then picked up on glass slides for light microscopy. An experienced pathologist blinded to the experiment evaluated all samples. All fields in each section were examined for grading of steatosis and necroinflammation according to the criteria described by Brunt *et al*[18].

The severity of steatosis was scored on the basis of the extent of involved parenchyma as 1 if fewer than 33% of the hepatocytes were affected, as 2 if 33%-66% of the hepatocytes were affected, as 3 if more than 66% of the hepatocytes were affected, and as 0 if no hepatocytes were affected.

Hepatic necroinflammation was graded from 0 to 3; score 1 (mild) = sparse or mild focal zone 3 hepatocyte injury/inflammation, score 2 (moderate) = noticeable zone 3 hepatocyte injury/inflammation, score 3 (severe) = severe zone 3 hepatocyte injury/inflammation, and score 0 = no hepatocyte injury/inflammation.

Statistical analysis

The data were expressed as mean ± SEM using the SPSS version 11.5 for Windows program. Statistical comparisons between groups were analyzed by ANOVA and post hoc comparisons were done with Bonferroni correction. $P < 0.05$ were considered significant.

RESULTS

Body mass and general condition

The body mass at 6 wk of the NASH group and NASH

Table 1 Body mass and serum biochemical parameters in all groups

Parameter (mean ± SEM)	Control (n = 8)	NASH (n = 8)	NASH + NAC_{20} (n = 9)
Body mass (g) at the beginning	239.0 ± 2.27	245.1 ± 1.0	251.4 ± 1.7
at 6 wk	438.4 ± 9.7	197.0 ± 8.1[a]	207.8 ± 6.9[a]
AST (U/L)	86.8 ± 4.3	53.6 ± 9.3[a]	65.6 ± 8.7
ALT (U/L)	40.2 ± 2.4	23.0 ± 1.9[a]	25.4 ± 5.7[a]
Cholesterol (g/L)	71.8 ± 1.8	94.8 ± 3.1[a]	91.4 ± 3.5[a]
Triglycerides (g/L)	90.3 ± 19.1	147.8 ± 32.6	89.2 ± 28.2

[a]$P < 0.05$ *vs* control.

Table 2 Effects of NAC on liver histology in rats with NASH (scores)

Group	n	Steatosis				Necroinflammation			
		0	1	2	3	0	1	2	3
Control	8	8	-	-	-	8	-	-	-
NASH	8	-	-	5	3	-	5	2	1
NASH + NAC_{20}	9	-	6	2	1	3	4	1	1

+ NAC_{20} group were decreased compared to the control (197.0 ± 8.1 g, 207.8 ± 6.9 g *vs* 438.4 ± 9.7 g, $P < 0.05$). Despite weight loss, the general condition of 100% fat diet-fed rats remained good throughout the observation periode. After the first 6 wk, rats were fed with regular dry rat chow for additional 4 wk. The body mass was significantly increased in all groups (Table 1).

Serum biochemical parameters

Serum biochemical parameters in the control and the experimental groups are given in Table 1. Serum AST and ALT activities decreased significantly in the NASH group when compared to the control group (AST; 53.7 ± 9.3 U/L *vs* 86.8 ± 4.3 U/L, ALT; 23.0 ± 1.9 U/L *vs* 40.1 ± 2.4 U/L, $P < 0.05$). Serum ALT but not AST activity returned to control levels in the NASH + NAC_{20} group (ALT 25.4 ± 5.7 U/L; AST 65.6 ± 8.7 U/L). Serum cholesterol was significantly higher in the NASH group and NASH + NAC_{20} group than that in the control group (94.8 ± 3.1 g/L, 91.4 ± 3.5 g/L *vs* 71.8 ± 1.8 g/L, $P < 0.05$), whereas there were no significant differences in serum triglycerides (Table 1).

Total glutathione level in whole blood

Whole blood total glutathione levels were significantly higher in the NASH group compared to the control group (2066.7 ± 93.8 μmol/L *vs* 1337.5 ± 31.5 μmol/L, $P < 0.05$). Glutathion in NASH + NAC_{20} group was significantly lower than in the NASH group (1394.8 ± 81.2 μmol/L *vs* 2066.7 ± 93.8 μmol/L, $P < 0.05$.

Hepatic MDA content

MDA was elevated significantly in the NASH group when compared to the control group (209.9 ± 43.8 μmol/g protein *vs* 3.8 ± 1.7 μmol/g protein, $P < 0.05$). There was no statistical significant difference in MDA levels in NASH + NAC_{20} group (150.1 ± 27.0 μmol/g protein).

Histopathological examination

Liver sections from rats fed with the regular dry rat chow had normal morphological appearance. In the NASH group, all animals developed moderate to severe macrovesicular steatosis, hepatocyte ballooning, mild to moderate inflammation, and regeneration of hepatocytes (Table 2). NAC treatment improved steatosis and necroinflammation scores in animals of the NASH + NAC_{20} group when compared with the NASH group (Figure 1).

DISCUSSION

Histopathology of NASH is similar to that of ethanol-induced hepatitis with the presence of macrovesicular steatosis, hepatocyte ballooning, necroinflammation, Mallory bodies, and fibrosis[1]. To study the pathogenesis of or therapeutic options for NASH, there are many models that can be used including a genetic model (obese rats), a model of methionine and choline deficient diet, a model of high fat liquid diet, and a 100% fat diet[5-8,15]. In this study, 100% fat diet was chosen to induce NASH in Sprague-Dawley rats as this procedure is fast, easy, and provides a comparable pattern of pathological changes as in humans although this model represents malnutrition induced steatohepatitis.

By feeding rats with 100% fat diet, the hepatic lesions of NASH were apparent within 6 wk. Histopathological examination showed macrovesicular steatosis, hepatocyte ballooning, Mallory bodies, and mild to moderate inflammation. One hundred percent fat diet caused mobilization of free fatty acid (FFA) from adipose tissue and transport into hepatocytes. In this condition, the liver failed to synthesize apolipoprotein that is required for packaging and exporting fat from the liver, triglycerides (TG) thus accumulate in the liver[19]. β-oxidation of FFA in hepatocytes produces reactive oxygen species (ROS) which activate lipid peroxidation[20]. ROS and lipid peroxidation cause direct damage to hepatocytes by disrupting membranes, protein, and DNA[21,22]. Hepatocyte damage and lipid peroxidation products induce an inflammatory response.

AST and ALT are useful screening tests for detecting liver injury[23]. They are found in hepatocytes and can not diffuse out of the cells in the physiological condition. When the hepatocyte is injured, plasma membrane can be disrupted and the leakage through extracellular fluid of the enzyme occurs where they can be detected at abnormal levels in the serum[24]. AST and ALT activities have been found to be increased in NASH rats[10,25-28]. In contrast, AST and ALT activities decreased significantly with 6 wk of 100% fat diet in this study. The decreased serum transaminases may be due to poor nutrition or hepatocyte death. Rats fed with 100% fat diet derived main energy from fat, when there were low in vitamin and mineral contents. The decreased AST and ALT levels were probably due to nutritional deficiency of pyridoxal phosphate which is a cofactor for both AST and ALT to catalyze the transfer of the α amino group from aspartate or alanine to α-ketoglutarate with made the release of pyruvate, oxaloacetate, and glutamate[23]. In addition,

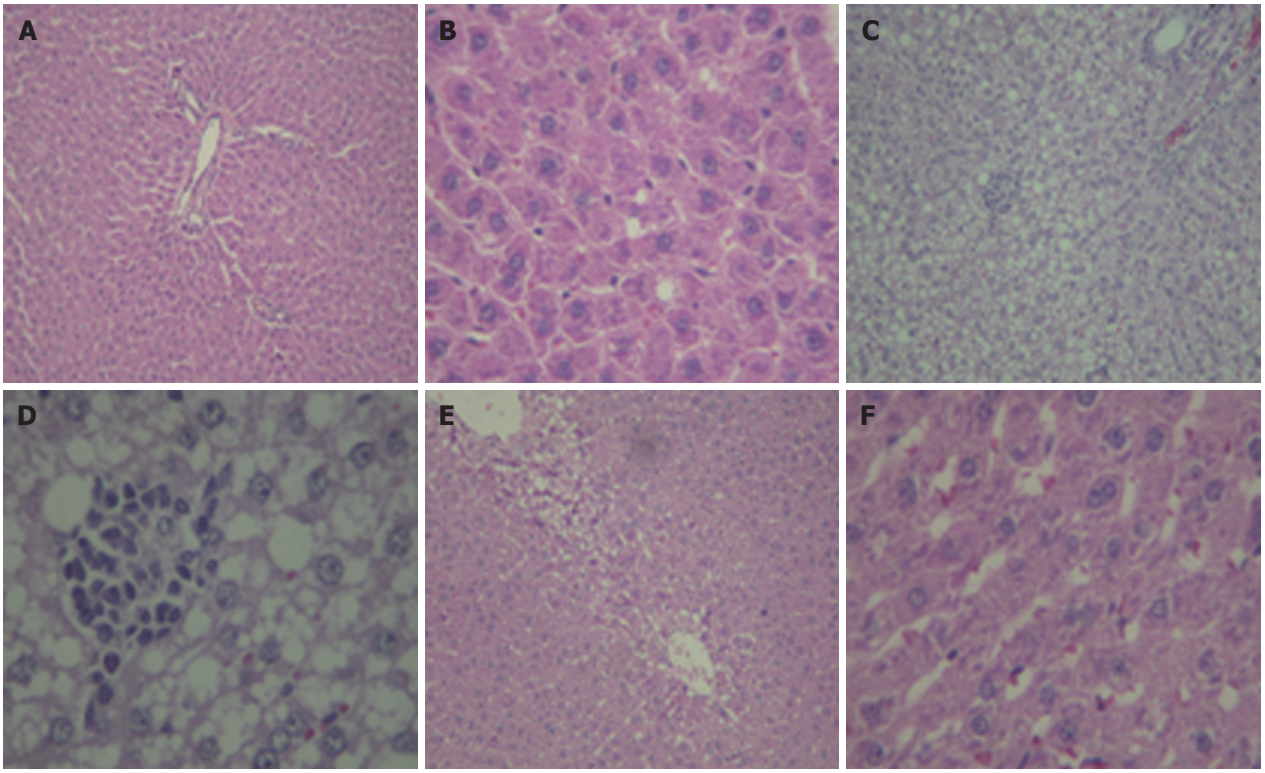


Figure 1 Hematoxylin and eosin staining of liver tissue. **A**, **B**: control; **C**, **D**: NASH, fed with 100% fat diet group showed macrovesicular steatosis, ballooning changes, Mallory bodies, hepatocyte necrosis, and infiltration of inflammatory cells; **E**, **F**: NASH + NAC_{20}, showed the improvement in steatosis and necroinflammation (**A**, **C**, **E**: x 10; **B**, **D**, **F**: x 40).

oxidative stress condition may be a cause of hepatocyte death, therefore, aminotransferases can not be produced.

In 100% fat diet-fed rats, body mass decreased significantly ($P < 0.05$) as compared to the control group. While serum cholesterol significantly increased, serum TG level was unchanged. Feeding with 100% fat diet for 6 wk caused a loss of body mass that may be due to a metabolic imbalance of carbohydrate, protein, and fat. Moreover, 100% fat diet contained highly saturated fat which may increase blood cholesterol concentration by 15% to 25%[29]. This result was from an increase of fat deposition in the liver which then provides the increased quantities of acetyl CoA in the liver cell for production of cholesterol[29]. The increased cholesterol was found in this experiment and had been observed in another study that used 10% lard oil and 2% cholesterol supplement adding into the standard diet[29].

FFA causes oxidative stress that has the potential to induce NASH[2]. FFA in the body is increased and this is associated with state of starvation[2]. Stored FFA can be mobilized from adipose tissue through lipolysis[2]. FFA metabolism increases the production of ROS which activated lipid peroxidation. Consequences are the disruption of membranes and the production of reactive metabolites such as MDA[20]. This study found high hepatic MDA levels in 100% fat-diet fed rats in accordance with studies by others[25-28]. Glutathione is the major intracellular non-protein antioxidant and plays a crucial role in the detoxification of free radicals[30,31]. Serum level of glutathione was increased in patients with NASH[32]. Similarly in this experiment, an increasing in total glutathione in whole blood with 100% fat diet feeding could be explained by compensatory protection mechanism against oxidative stress.

NAC is a thiol compound that acts directly as free radical scavenger and as a precursor of reduced glutathione[33]. Therefore, treatment with 20 mg/kg of NAC improved the total glutathione level to normal level in NASH + NAC_{20} group and improved necroinflammation score. Because of some limitations of our study, such as dose of NAC, time for treatment, and the number of animals, the effect of NAC on reducing hepatic MDA level remained unclear. In our previous study, diet treatment alone and diet plus NAC groups, total glutathione, serum AST, ALT, cholesterol, TG, and hepatic MDA returned to normal levels as in the control group. In addition, the pathological changes of liver in these groups were improved[14]. These results emphasized how crucial the nutritional composition of the diet is. Good proportion of nutrients (i.e., carbohydrate, lipid, and protein) is essential for growth and maintenance. These nutrients supply energy, promote growth, repair body tissues, and regulate metabolic processes[34].

In conclusion, feeding with 100% fat diet for 6 wk induced macrovesicular steatosis, hepatocyte ballooning, and inflammation in rats similar to histopathology of NASH. Treatment with NAC in NASH could improve oxidative stress and liver histopathology.

COMMENTS

Background

Non-alcoholic steatohepatitis (NASH), in advanced stages, can cause liver fibrosis, eventually progressing to cirrhosis in some patients. Oxidative stress is believed

to play an important role in pathogenesis of NASH. N-acetylcysteine (NAC) is a glutathione precursor which increases glutathione levels in hepatocytes. Increased glutathione levels, in turn, limit the production of reactive oxygen species (ROS) which cause hepatocellular injury that could protect hepatocytes from oxidative stress.

Research frontiers

NAC is a thiol compound that acts directly as free radical scavenger. In the pathogenesis of NASH, prevention of oxidative stress could protect hepatocytes from injury. The hotspots of this study indicate that NAC treatment could attenuate oxidative stress and improve liver histology in rats with NASH.

Innovations and breakthroughs

According to a previous report, oral NAC treatment of NASH patients for several months was found to significantly improve aminotransferase levels. However, the mechanism remained unclear. This study is a novel and well conducted experimental study showing the efficacy of NAC on improvement of total glutathion level and hepatic MDA in rats with NASH. Furthermore, treatment with NAC showed improvement in steatosis and necroinflammation.

Applications

Our data indicate that NAC treatment could attenuate oxidative stress and improve liver histology in rats with NASH.

Terminology

NASH is a liver disease characterized by macrovesicular steatosis, hepatocyte necrosis, inflammation, Mallory bodies, and fibrosis. In initial phases, during which fat accumulates in the liver, no clinical symptoms are evident. In advanced stages, fibrosis is detectable, eventually progressing to cirrhosis. NAC is a glutathione precursor which increases glutathione levels in hepatocytes. Increased glutathione levels, in turn, limit the production of ROS which cause hepatocellular injury.

Peer review

This is an experimental work on a steatosis model in the rat, induced by 100% fat diet in which the co-administration of NAC protects against fat induced liver injury. This is a very interesting and well conducted experimental study showing the efficacy of NAC in preventing biochemical and histological alterations secondary to a fat rich diet.

REFERENCES

1 **Chalasani N**, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2004; **99**: 1497-1502

2 **Te Sligte K**, Bourass I, Sels JP, Driessen A, Stockbrugger RW, Koek GH. Non-alcoholic steatohepatitis: review of a growing medical problem. *Eur J Intern Med* 2004; **15**: 10-21

3 **DeFronzo RA**. Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidaemia and atherosclerosis. *Neth J Med* 1997; **50**: 191-197

4 **Medina J**, Fernández-Salazar LI, García-Buey L, Moreno-Otero R. Approach to the pathogenesis and treatment of nonalcoholic steatohepatitis. *Diabetes Care* 2004; **27**: 2057-2066

5 **Campfield LA**, Smith FJ, Burn P. The OB protein (leptin) pathway--a link between adipose tissue mass and central neural networks. *Horm Metab Res* 1996; **28**: 619-632

6 **Weltman MD**, Farrell GC, Liddle C. Increased hepatocyte CYP2E1 expression in a rat nutritional model of hepatic steatosis with inflammation. *Gastroenterology* 1996; **111**: 1645-1653

7 **Koteish A,** Diehl AM. Animal models of steatosis. *Semin Liver Dis* 2001; **21**: 89-104

8 **Lieber CS**, Leo MA, Mak KM, Xu Y, Cao Q, Ren C, Ponomarenko A, DeCarli LM. Model of nonalcoholic steatohepatitis. *Am J Clin Nutr* 2004; **79**: 502-509

9 **García-Ruiz C**, Colell A, Morales A, Kaplowitz N, Fernández-Checa JC. Role of oxidative stress generated from the mitochondrial electron transport chain and mitochondrial glutathione status in loss of mitochondrial function and activation of transcription factor nuclear factor-kappa B: studies with isolated mitochondria and rat hepatocytes. *Mol Pharmacol* 1995; **48**: 825-834

10 **Robino G**, Parola M, Marra F, Caligiuri A, De Franco RM, Zamara E, Bellomo G, Gentilini P, Pinzani M, Dianzani MU. Interaction between 4-hydroxy-2,3-alkenals and the platelet-derived growth factor-beta receptor. Reduced tyrosine phosphorylation and downstream signaling in hepatic stellate cells. *J Biol Chem* 2000; **275**: 40561-40567

11 **Gulbahar O**, Karasu A, Ersoz G, Akarca US, Musoglu A. Treatment of non-alcoholic steatohepatitis with N- acetyl cysteine. *Gastroenterology* 2000; **118**: A1444

12 **Pastor A**, Collado PS, Almar M, González-Gallego J. Antioxidant enzyme status in biliary obstructed rats: effects of N-acetylcysteine. *J Hepatol* 1997; **27**: 363-370

13 **Gulbahar O**, Karasu A, Ersoz G, Akarca US, Musoglu A. N-acetyl cysteine in the treatment of non-alcoholic steatohepatitis. *J Gastroenterology* 2003; **18**: 1220-1221

14 **Samuhasaneeto S**, Thong-Ngam D, Kulaputana O, Patumraj S, Klaikeaw N. Effects of N-acetylcysteine on oxidative stress in rats with non-alcoholic steatohepatitis. *J Med Assoc Thai* 2007; **90**: 788-797

15 **Thong-Ngam D**, Samuhasaneeto S, Suyasunanont D, Wisedopas N. Development of a simple rat model of nonalcoholic steatohepatitis. *Thai J Gastroenterol* 2005; **6**: 144-148

16 **Ohkawa H**, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; **95**: 351-358

17 **Lowry oh**, rosebrough nj, farr al, randall rj. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; **193**: 265-275

18 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474

19 **Brody T**. Nutritional biochemistry. 2nd ed. The United States: Academic Press, 1994: 243-245

20 **Benzie IF**. Lipid peroxidation: a review of causes, consequences, measurement and dietary influences. *Int J Food Sci Nutr* 1996; **47**: 233-261

21 **de Knegt RJ**. Non-alcoholic steatohepatitis: clinical significance and pathogenesis. *Scand J Gastroenterol Suppl* 2001; **234**: 88-92

22 **Day CP**. Pathogenesis of steatohepatitis. *Best Pract Res Clin Gastroenterol* 2002; **16**: 663-678

23 **Kaplowitz N**. Liver and biliary diseases. Baltimore: Williams & Wilkins, 1992: 383

24 **Robbins SL**. Pathologic basic of disease. London: W. B. Soder Company, 1974: 25-30

25 **Fan JG**, Zhong L, Xu ZJ, Tia LY, Ding XD, Li MS, Wang GL. Effects of low-calorie diet on steatohepatitis in rats with obesity and hyperlipidemia. *World J Gastroenterol* 2003; **9**: 2045-2049

26 **Leclercq IA**, Farrell GC, Field J, Bell DR, Gonzalez FJ, Robertson GR. CYP2E1 and CYP4A as microsomal catalysts of lipid peroxides in murine nonalcoholic steatohepatitis. *J Clin Invest* 2000; **105**: 1067-1075

27 **Kirsch R**, Clarkson V, Shephard EG, Marais DA, Jaffer MA, Woodburne VE, Kirsch RE, Hall Pde L. Rodent nutritional model of non-alcoholic steatohepatitis: species, strain and sex difference studies. *J Gastroenterol Hepatol* 2003; **18**: 1272-1282

28 **George J**, Pera N, Phung N, Leclercq I, Yun Hou J, Farrell G. Lipid peroxidation, stellate cell activation and hepatic fibrogenesis in a rat model of chronic steatohepatitis. *J Hepatol* 2003; **39**: 756-764

29 **Guyton AC**, Hall JE. Textbook of Medical physiology. 10th ed. The United States W. B. Saunders, 2000: 788

30 **Meister A**, Larsson A. Glutathione synthetase deficiency and other disorders of the γ-glutamyl cycle. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic basis of inherited disease. New York McGraw-Hill, 1989; 855-868

31 **Hayes JD**, McLellan LI. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free Radic Res* 1999; **31**: 273-300
32 **Koruk M**, Taysi S, Savas MC, Yilmaz O, Akcay F, Karakok M. Oxidative stress and enzymatic antioxidant status in patients with nonalcoholic steatohepatitis. *Ann Clin Lab Sci* 2004; **34**: 57-62
33 **Cotgreave IA**. N-acetylcysteine: pharmacological considerations and experimental and clinical applications. *Adv Pharmacol* 1997; **38**: 205-227
34 **Guthrie HA**. Introductory nutrition. 6th ed. St Louis: Times Mirror/Mosby College Publishing, 1986: 11

S- Editor Ma N **L- Editor** Mihm S **E- Editor** Yin DH

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5133-5138
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Risk factors for lymph node metastasis and evaluation of reasonable surgery for early gastric cancer

Ying-Ying Xu, Bao-Jun Huang, Zhe Sun, Chong Lu, Yun-Peng Liu

Ying-Ying Xu, Yun-Peng Liu, Department of Medical Oncology, First Hospital of China Medical University, Shenyang 110001, Liaoning Province, China
Bao-Jun Huang, Zhe Sun, Chong Lu, Department of Surgical Oncology, First Hospital of China Medical University, Shenyang 110001, Liaoning Province, China
Supported by the National Natural Science Foundation of China, No. 30370640
Correspondence to: Yun-Peng Liu, Department of Medical Oncology, First Hospital of China Medical University, Shenyang 110001, Liaoning Province, China. lovecmustar@163.com
Telephone: +86-13940521129 Fax: +86-24-22703576
Received: May 15, 2007 Revised: August 11, 2007

Abstract

AIM: To give the evidence for rationalizing surgical therapy for early gastric cancer with different lymph node status.

METHODS: A series of 322 early gastric cancer patients who underwent gastrectomy with more than 15 lymph nodes retrieved were reviewed in this study. The rate of lymph node metastasis was calculated. Univariate and multivariate analyses were performed to evaluate the independent factors for predicting lymph node metastasis.

RESULTS: No metastasis was detected in No.5, 6 lymph nodes (LN) during proximal gastric cancer total gastrectomy, and in No.10, 11p, 11d during for combined resection of spleen and splenic artery and in No.15 LN during combined resection of transverse colon mesentery. No.11p, 12a, 14v LN were proved negative for metastasis. The global metastastic rate was 14.6% for LN, 5.9% for mucosa, and 22.4% for submucosa carcinoma, respectively. The metastasis in group Ⅱ was almost limited in No.7, 8a LN. Multivariate analysis identified that the depth of invasion, histological type and lymphatic invasion were independent risk factors for LN metastasis. No metastasis from distal cancer (≤ 1.0 cm in diameter) was detected in group Ⅱ LN. The metastasis rate increased significantly when the diameter exceeded 3.0 cm. All tumors (≤ 1.0 cm in diameter) with LN metastasis and mucosa invasion showed a depressed macroscopic type, and all protruded carcinomas were > 3.0 cm in diameter.

CONCLUSION: Segmental/subtotal gastrectomy plus D_1/D_1 + No.7 should be performed for carcinoma (≤ 1.0 cm in diameter, protruded type and mucosa invasion). Subtotal gastrectomy plus D_2 or D_1 + No.7, 8a, 9 is the most rational operation, whereas No.11p, 12a, 14v lymphadenectomy should not be recommended routinely for poorly differentiated and depressed type of submucosa carcinoma (> 3.0 cm in diameter). Total gastrectomy should not be performed in proximal, so does combined resection or D_2^+/D_3 lymphadenectomy.

Key words: Lymph node; Metastasis; Surgery; Early gastric cancer

Xu YY, Huang BJ, Sun Z, Lu C, Liu YP. Risk factors for lymph node metastasis and evaluation of reasonable surgery for early gastric cancer. *World J Gastroenterol* 2007; 13(38): 5133-5138

http://www.wjgnet.com/1007-9327/13/5133.asp

INTRODUCTION

Gastric cancer is still one of the important causes of cancer-related death in China. Although early gastric cancer accounts for less than 10% of gastric cancers, excellent outcome of surgery has been reported, with a 5-year survival rate higher than 90%[1-3]. In the past 20 years, most surgeons considered D_2 lymphadenectomy the standard and optimal surgical procedure for patients with early gastric cancer. Even total gastrectomy and D_3 lymphadenectomy with combined resection of other organs have been used to achieve curative (R_0) resection[3-9]. The lymph node metastasis rate of early gastric cancer is reported to be 11%-18% and 70%-80% patients will undergo over-surgery with D_2 lymphadenectomy[1,4,8,9]. Consequently, investigating the risk factors for lymph node metastasis is the key to rational surgery of early gastric cancer, which may improve the 5-year survival rate of patients and their quality of life. This study was to retrospectively analyze the location, frequency, degree of and risk factors for lymph node metastasis in 322 patients with early gastric cancer, in order to rationalize surgical therapy.

MATERIALS AND METHODS

Patients

Between February 1972 and August 2006, a consecutive series of 322 early gastric cancer patients underwent

Table 1 Site and frequency of lymph node metastasis of cancer in the lower and middle thirds of stomach

The lower third of the stomach	Frequency of LNM		The middle third of the stomach	Frequency of LNM	
	Possitive	%		Possitive	%
Group 1			Group 1		
No.3	12	4.9	No.1	3	4.8
No.4	16	6.5	No.3	6	9.7
No.5	3	1.2	No.4	0	0.0
No.6	14	5.7	No.5	1	1.6
			No.6	1	1.6
Total	35	14.3	Total	8	12.9
Group 2			Group 2		
No.1	1	0.4	No.7	3	4.8
No.7	13	5.3	No.8a	1	1.6
No.8a	7	2.9	No.9	0	0.0
No.9	2	0.8	No.11p	0	0.0
No.11p	0	0.0	No.12a	0	0.0
No.12a	0	0.0			
No.14v	0	0.0			
Total	18	7.3	Total	4	6.5

LNM: Lymph node metastasis; No.1: Right paracardial LN; No.3: LN along the lesser curvature; No.4: LN along the greater curvature; No.5: suprapyloric LN; No.6: Infrapyloric LN; No.7: LN along the left gastric artery; No.8a: LN along the common hepatic artery (anterosuperior group); No.9: LN around the celiac artery; No.11p: LN along the proximal splenic artery; No.12a: LN in the hepatoduodenal ligament (along the hepatic artery); No.14v: LN along the superior mesenteric vein.

gastrectomy at the Department of Oncologic Surgery, First Affiliated Hospital, China Medical University. The patients (242 men and 80 women) ranged in age from 19 to 80 (53.8 ± 12.3) years participated in the study. Early gastric cancer was located in the lower third of stomach (L/LM) of 145 patients, in the middle third (M/ML/MU) of 14 patients, in the upper third (U/UM) of 11 patients, and in whole stomach (UML) of 4 patients. A total of 292 patients underwent distal gastrectomy, 10 proximal gastrectomy, 20 total gastrectomy, 2 combined resection of spleen, and 3 combined resection of transverse colon mesentery. D_1 lymphadenectomy was performed in 57 patients; D_1 + No.7 in 58 patients; D_1 + No.7, 8a, 9 in 63 patients; D_2 in 107 patients; and D_2^+ or D_3 in 37 patients. The methods of pathology diagnosis, lymph node grouping and surgery have been described previously[10].

Pathology

Serial section of specimens was performed for an accuracy pathological diagnosis. Mucosa carcinoma was diagnosed in 152 patients and submucosa carcinoma in 170 patients based on the depth of invasion. Protruded type was found in 23 patients, flat type in 38 patients, and depressed type in 263 patients, respectively in the light of macroscopic appearance. The tumor diameter ranged from 0.4 to 14.0 (3.2 ± 1.8) cm. Well or moderately differentiated tumor was found in 142 patients, and poorly differentiated tumor in 180 patients according to their histological type. Mass type was observed in 102 patients, nest type in 91 patients and diffused type in 129 patients, respectively, based on the histological growth pattern. Lymphatic vessel invasion occurred in 19 patients.

Statistical analysis

All data were analyzed by SPSS11.5. The correlation between clinicopathological factors and nodal involvement was evaluated by univariate analysis. Multivariate analysis was performed to evaluate the independent factors for predicting lymph node metastasis. $P < 0.05$ was considered statistically significant.

RESULTS

Evaluation of lymphadenectomy

Five patients underwent combined resection of other organs due to misdiagnosis as advanced gastric carcinoma. Depressed type of mucosa and submucosa carcinoma was diagnosed in 2 and 3 patients, respectively. Proximal gastric cancer without metastasis in No.10, 11p, 11d lymph nodes was diagnosed in 2 patients undergoing combined resection of spleen and splenic artery. Distal gastric cancer without metastasis in No.15 lymph node was diagnosed in 3 patients undergoing combined resection of transverse colon mesentery. No.5, 6 lymph nodes were negative in 20 patients with proximal gastric cancer after total gastrectomy.

Metastasis of distal gastric cancer was found in 2 out of the 37 patients undergoing extended lymphadenectomy (> D_2). Depressed type of mucosa and submucosa carcinoma was found in the 2 patients. Metastasis of group I lymph nodes was detected both in 285 patients with middle and distal gastric cancer who underwent ≤ D_2 lymphadenectomy. The detection rate was 12.9% and 14.3%, respectively. A frequent metastasis of No.7, 8a lymph nodes (6.5%-8.2%) and an occasional metastasis of No.9 lymph nodes were also found. No metastasis was detected in No.11, 12a, 14v lymph nodes (Table 1).

Relationship between frequency of lymph node metastasis and location of focus

The number of retrieved lymph nodes in all patients was more than 15, ranging from 15 to 75 (median, 16). Lymph node metastasis was detected in 47 patients (14.6%), and the number of metastatic lymph nodes ranged from 1 to 16 (median, 2). Of the patients with lymph node metastasis, 33 were male (13.6%), 14 female (17.5%). Distal gastric cancer was diagnosed in 38 patients (15.5%), middle gastric cancer in 9 (14.5%), protruded type in 3 patients (14.3%), flat type in 4 patients (10.5%),and depressed type in 40 patients (15.2%). The diameter of tumor was ≤ 1.0 cm in 5 patients (19.5%), 1.1-2.0 cm in 13 patients (11.2%), 2.1-3.0 cm in 6 patients (10.0%), > 3.0 cm in 23 patients (19.2%). Mucosa carcinoma was found in 9 patients (2.9%), submucosa carcinoma in 38 patients (22.4%), poorly differentiated carcinoma in 35 patients (19.4%), well or moderately differentiated in 12 patients (8.5%), mass type in 15 patients (14.7%), nest type in 12 patients (13.2%), diffused type in 20 patients (15.5%), negative lymphatic invasion in 36 patients (57.9%), and positive lymphatic invasion in 11 patients (11.9%).

Of the 245 patients with distal gastric cancer, 35 had metastasis in group I lymph nodes (14.3%), and 18 in group Ⅱ lymph nodes (7.3%). Metastasis was detected in all group

Table 2 Comparison of clinicopathological features between patients with and without lymph node metastasis

Factors	Node negative	Node positive	*P* value
Dissected nodes (mean ± SD)	19.3 ± 7.5	20.8 ± 6.9	0.188
Age, yr (mean ± SD)	53.9 ± 12.1	52.9 ± 13.2	0.606
Tumor maximum diameter (cm, mean ± SD)	3.1 ± 1.8	3.5 ± 1.8	0.197
Gender			
Male	209	33	
Female	66	14	0.465
Tumor location			
Upper	11	0	
Middle	53	9	
Lower	207	38	0.435
Total	4	0	
Depth of invasion			
Mucosa	143	9	
Submucosa	132	38	< 0.001
Histological type			
Differentiated	130	12	
Undifferentiated	145	35	0.007
Macroscopic type			
Protruded	18	3	
Flat	34	4	0.746
Depressed	223	40	
Growth manner			
Mass	87	15	
Nest	79	12	0.891
Diffuse	109	20	
lymphatic invasion			
Negative	267	36	
Positive	8	11	< 0.001

Differentiated: Papillary and tubular adenocarcinoma; Undifferentiated: Poorly differentiated adenocarcinoma and signet-ring cell carcinoma; Protruded: Ⅰ and Ⅱa; flat: Ⅱb; depressed: Ⅱc and Ⅲ.

Table 3 Logistic regression analysis for variables associated with lymph node metastasis in EGC

Explanatory variables	Odds ratio	95% CI	*P* value
Depth of invasion	3.67	1.62-8.30	0.002
Histological type	3.39	1.39-8.27	0.007
Lymphatic invasion	8.41	2.86-24.74	< 0.001
Tumor maximum diameter	1.23	1.0-1.49	0.042
Gender	1.10	0.46-2.21	0.981
Age	0.99	0.97-1.03	0.726
Tumor location	0.60	0.29-1.23	0.161
Growth manner	0.58	0.26-1.30	0.186
Macroscopic type	1.14	0.56-2.34	0.715

CI: Confidence interval. Depth of invasion, histological type, lymphatic invasion and tumor maximum diameter are the independent risk factors correlated with lymph node involvement.

Ⅰ lymph nodes with a frequency of 5.7% for No.6, 6.5% for No.4, 4.9% for No.3, and 1.2% for No.5, respectively. In group Ⅱ lymph nodes, the most frequent metastasis was detected in No.7 lymph nodes (5.3%) and No.8a lymph nodes (2.9%), and less frequent metastasis in No.9 lymph nodes (0.8%), No.1 lymph nodes (0.4%). Metastasis of group Ⅲ lymph nodes was detected in only 2 patients.

Of the 62 patients with middle gastric cancer, 8 had metastasis of group Ⅰ lymph nodes (12.9%), and 4 had metastasis of group Ⅱ lymph nodes (6.5%). The rate of metastasis of group Ⅰ lymph nodes was 9.7% for No.3, 4.8% for No.1, 1.6% for No.5, and 1.6% for No.6, respectively. Metastasis of No.7 (4.8%) and No.8a (1.6%) lymph nodes was detected in group Ⅱ lymph nodes. No lymph node metastasis was detected in the 11 patients with proximal gastric cancer.

Risk factors correlated with lymph node involvement

In this series, the mean number of retrieved lymph nodes was 19.3 ± 7.5 in patients without lymph node metastasis, and 20.8 ± 6.9 in patients with lymph node metastasis. The difference was not significant ($F = 1.741$, $P = 0.188$). The univariate analysis showed that three variables were significantly indicative of lymph node metastasis: depth of invasion, histological type, and lymphatic invasion ($P < 0.001$). The lymph node metastasis rate of poorly differentiated submucosa carcinoma with positive lymphatic invasion was significantly higher than that of well differentiated mucosa carcinoma with negative lymphatic invasion (Table 2).

The multivariant analysis showed that all the variables (depth of invasion, histological type, and lymphatic invasion) remained significant, indicating that the independent risks were correlated with lymph node involvement. The lymph node metastasis of submucosa carcinoma was 3.7 times higher than that of mucosa carcinoma. The lymph node metastasis of poorly differentiated carcinoma was 3.4 times higher than that of well differentiated carcinoma. The positive lymphatic invasion was 8.4 times higher than that of negative lymphatic invasion. The maximum diameter of the tumor was also an important variable correlated with lymph node involvement (OR = 1.23, $P = 0.042$), the rate of lymph node metastasis was associated with the maximum tumor diameter (Table 3).

Relationship between lymph node metastasis and clinicopathological factors for distal gastric cancer

Metastasis of distal gastric cancer hardly went beyond group Ⅰ lymph nodes when the maximum tumor diameter was ⩽ 1.0 cm. Corresponding to the increased maximum diameter, the rate of metastasis in group Ⅱ lymph nodes was increased, but often limited in No7, 8a lymph nodes. Only when the maximum tumor diameter was > 3.0 cm, could metastasis of No.1 and 9 lymph nodes be detected. Compared with protruded type of carcinoma, in which metastasis could only be detected in group Ⅰ lymph nodes, depressed type of carcinoma often had combined metastasis in both group Ⅰ and Ⅱ lymph nodes, and the number of metastasis lymph nodes would increase significantly. Metastasis of mucosa carcinoma was detected in all group Ⅰ lymph nodes. Metastasis of poorly differentiated and depressed types of mucosa carcinoma in No.1 lymph nodes was detected in only 1 patient, whose tumor diameter was > 3.0 cm. Metastasis of submucosa carcinoma was detected in both group Ⅰ and Ⅱ lymph nodes, and the rate of lymph node metastasis was significantly higher than that of mucosa carcinoma ($P < 0.01$). Metastasis of poorly and well differentiated carcinoma was detected in group Ⅰ and Ⅱ lymph nodes, but the metastasis rate of poorly differentiated

Table 4 Depth of invasion, histological type, macroscopic type, lymphatic penetration of lymph node metastasis and tumor maximum diameter in the lower third of stomach

Factors	Group 1 metastasis (person)						Group 2 metastasis (person)					
	No.3	No.4	No.5	No.6	Total	*P* value	No.1	No.7	No.8a	No.9	Total	*P* value
Tumor maximum diameter (cm)												
≤ 1.0	1	0	0	3	4		0	0	0	0	0	
1.1-2.0	4	4	2	4	11		0	3	3	0	5	
2.1-3.0	0	2	1	0	3		0	1	0	0	1	
> 3.0	7	10	0	7	17	0.143	1	9	4	2	12	0.018
Histological type												
Diff	4	4	0	4	8		0	2	0	1	2	
Undiff	8	12	3	10	27	0.025	1	11	7	1	16	0.006
Depth of invasion												
M	1	4	2	3	9		1	0	0	0	1	
Sm	11	12	1	11	26	0.010	0	13	7	2	17	< 0.001
Growth manner												
Mass	5	5	0	5	10		0	4	3	1	7	
Nest	3	3	2	2	8	0.669	0	3	2	1	4	0.689
Diffuse	4	8	1	7	17		1	6	2	0	7	
Macroscopic type												
Protruded	0	2	0	0	2		0	0	0	0	0	
Flat	1	2	0	1	3		0	2	0	0	2	
Depressed	11	12	3	13	30	0.819	1	11	7	2	16	0.501
Lymphatic invasion												
Negative	10	13	2	11	27		1	8	6	2	13	
Positive	2	3	1	3	8	< 0.001	0	5	1	0	5	< 0.001

Diff: Differentiated histological type; Undiff: Undifferentiated histological type; M: Mucosa; Sm: Submucosa.

carcinoma was significantly higher than that of well differentiated carcinoma ($P < 0.05$). The metastasis rate of positive lymphatic invasive carcinoma was also significantly higher than that of negative lymphatic invasive carcinoma ($P < 0.01$) (Table 4).

DISCUSSION

Since 1980's, D_2 lymphadenectomy has been widely accepted as the standard surgery for early gastric cancer, especially for submucosa carcinoma. However, it was reported that D_2 lymphadenectomy does not increase the long-term survival of patients, compared with D_1 or D_1^+ lymphadenectomy[7-9,11]. Since lymph node metastasis remains one of the most important predictors for survival, reduction in lymphadenectomy will probably result in residue of metastatic lymph nodes. Unnecessarily extended resection will induce a series of complications, which also result in a poor quality of life. Thus, it is important to standardize the optimal extent of lymph node dissection by investigating lymph node metastasis of early gastric cancer.

In the present study, total gastrectomy for carcinoma in the upper third of stomach was selected according to the status of No.5 and 6 lymph nodes. Metastasis was not detected in all proximal gastric cancer patients after total gastrectomy. Considering the complications after total gastrectomy, it was not used as a routine operation for proximal early gastric cancer. Furthermore, metastasis was not detected in No.10, 11p, 11d lymph nodes after combined resection of spleen or splenic artery and in No.15 lymph nodes after combined resection of transverse colon mesentery. Therefore, we conclude that combined resection of other organs should not be performed in early gastric cancer. In our study, most patients undergoing combined resection were due to adhesion of serosa and transverse colon mesentery or due to inflammatory swelling of inflammatory splenic hilum lymph nodes. Thus, estimating the depth of invasion accurately is the key to optimal surgery. Endoscopic ultrasonography and three-dimensional spiral CT (3DCT) provide more accurate information on the depth of invasion. It was reported that 70%-80% of early gastric cancers can be diagnosed by endoscopic ultrasonography combined with three-dimensional CT[12-14].

Whether D_2 lymphadenectomy for early gastric cancer should be performed remains controversial. Many researchers consider D_1 + No.7, 8a or D_1 + No.7, 8a, 9 lymphadenectomy as the standard operation for most of early gastric cancers[15-17]. In our series, no metastasis of middle and distal gastric cancer was detected in No. 11p, 12a, 14v lymph nodes, suggesting that neither dissection of No. 11p, 12a, 14v lymph nodes nor D_2^+/D_3 lymphadenectomy is necessary for distal early gastric cancer.

It was reported that the lymph node metastasis rate is 0%-3% for mucosa carcinoma and 20% for submucosa carcinoma. It has been shown that 9%-16% of metastatic lymph nodes are detected in group Ⅰ, 4%-6% in group Ⅱ, and 0.3%-1% in group Ⅲ[16-18]. In this study, the metastasis rate in group I and Ⅲ lymph nodes is consistent with the reported data. The metastasis rate in group Ⅱ was 7.3% for distal cancer and 6.5% for middle cancer, higher than that in former studies.

It has been widely accepted that the status of lymph node involvement is closely correlated with the depth of invasion, but the relationship between metastasis and focus diameter, histological type and macroscopic type is still controversial[8,16,17,19-21]. Kunisaki *et al*[17] reported that mucosa carcinoma (> 3.0 cm in diameter) has a

higher lymph node involvement, while there is no distinct correlation between the diameter, macroscopic type, histological type and metastasis of submucosa carcinoma. Shimoyama *et al*[16] found that the metastasis rate for intestinal submucosa carcinoma (≤ 1.5 cm in diameter) and diffused submucosa carcinoma r (≤ 1.0 cm in diameter) is very low (3%), and limited in group I lymph nodes. If the diameter went beyond the former cut-point, the rate of metastasis would increase significantly and No.7, 8a, 9 lymph nodes (2.3%) could be detected even in group Ⅱ lymph nodes. Gotoda *et al*[20] reported that lymph node metastasis of well differentiated mucosa carcinoma (≤ 3.0 cm in diameter) is seldom detected. Whereas lymph node metastasis of submucosa carcinoma (> 3.0 cm in diameter) with positive lymphatic invasion increase significantly.

In our study, no metastasis of distal gastric cancer (≤ 1.0 cm in diameter) with mucosa invasion was detected in group Ⅱ lymph nodes. The rate of metastasis in group Ⅱ lymph nodes significantly increased when the tumor diameter was > 3.0 cm. All the carcinomas (≤ 1.0 cm in diameter and/or mucosa invasion) with lymph node involvement showed depressed macroscopic type. The diameter of protruded carcinoma with lymph node involvement was > 3.0 cm. Multivariate analysis showed that the depth of invasion, histological type and lymphatic penetration were independent risk factors for lymph node metastasis and the maximum diameter was also an important factor. Our study also revealed the relationship between metastasis of distal gastric cancer in group Ⅱ lymph nodes and the diameter, histological type, depth of invasion and lymphatic invasion of the tumor. The rate of involved lymph nodes significantly increased compared with poorly differentiated, sub-mucosa carcinoma and positive lymphatic invasion, suggesting that the following high risk factors for lymph node metastasis of distal gastric cancer in group Ⅱ lymph nodes are tumor diameter > 3.0 cm, depressed type, poorly differentiated submucosa carcinoma and positive lymphatic invasion. Standard D_2 lymphadenectomy should be performed to achieve curative (R_0) resection in such patients.

In summary, selection of reasonable surgery for early gastric cancer should be based on the pathobiologic behaviour of the tumor. Segmental/subtotal gastrectomy plus D_1/D_1 + No.7 lymphadenectomy should be performed for carcinoma (≤ 1.0 cm in diameter, protruded type and mucosa invasion). Subtotal gastrectomy plus D_2 or D_1 + No.7, 8a, 9 lymphadenectomy is the most rational operation for poorly differentiated and depressed type of submucosa carcinoma (> 3.0 cm in diameter), whereas No.11p, 12a, 14v lymphadenectomy should not be performed routinely. Total gastrectomy should not be performed for proximal gastric cancer. Combined resection of other organs or D_2^+/D_3 lymphadenectomy should always be avoided.

COMMENTS

Background

Since 1980's, D_2 lymphadenectomy has been widely accepted as the standard surgery for early gastric cancer, especially for submucosa carcinoma. Even total gastrectomy and D_3 lymphadenectomy with combined resection of other organs have been used to achieve curative (R_0) resection. However, it was reported that application of extended surgery does not reasonably increase the long-term survival of patients.

Research frontiers

It has been widely accepted that the status of lymph node involvement is closely correlated with the depth of invasion, but the relationship between metastasis and diameter of focus, histological type and macroscopic type is still controversial. Since lymph node metastasis remains one of the most important predictors for survival, reduction in lymphadenectomy will probably result in residue of metastatic lymph nodes. Unecessarily extended resection will induce a series of complications, which also result in a poor quality of life.

Innovations and breakthroughs

The results of this study show that depth of invasion, histological type and lymphatic invasion are independent risk factors for lymph node metastasis. There is a relationship between the pathobiologic behavior of tumor and reasonable surgery for early gastric cancer.

Applications

With the knowledge of the risk factors and rules of lymph node metastasis, clinical doctors can select surgery for early gastric cancer more reasonably.

Terminology

D lymphadenectomy: Lymph node dissection according to the Japanese Gastric Cancer Association (JGCA) criteria. Lymph node dissection is divide into D_1 lymphadenectomy, D_2 lymphadenectomy and D_3 lymphadenectomy.

Peer review

The authors rationalized surgical therapy for early gastric cancer with different lymph node status and suggest that segmental/subtotal gastrectomy plus D_1/D_1 + No.7 should be performed for carcinoma (≤ 1.0 cm in diameter, protruded type and mucosa invasion). Subtotal gastrectomy plus D_2 or D_1 + No.7, 8a, 9 is the most rational operation for poorly differentiated and depressed type of submucosa carcinoma (> 3.0 cm in diameter), whereas No.11p, 12a, 14v lymphadenectomy should not be recommended routinely. Total gastrectomy or D_2^+/D_3 lymphadenectomy should not be performed in proximal and combined resection.

REFERENCES

1 **Roviello F,** Rossi S, Marrelli D, Pedrazzani C, Corso G, Vindigni C, Morgagni P, Saragoni L, de Manzoni G, Tomezzoli A. Number of lymph node metastases and its prognostic significance in early gastric cancer: a multicenter Italian study. *J Surg Oncol* 2006; **94**: 275-280; discussion 274

2 **Shan JX,** Chen JQ, Wang SB. Recurrence of early gastric cancer. *Zhonghua Yixue Zazhi* 1996; **76** (10): 750-755

3 **Ikeda Y,** Saku M, Kawanaka H, Nonaka M, Yoshida K, Maehara Y, Sugimachi K. Prophylactic lymph node dissection for early gastric cancer invading submucosa. *Hepatogastroenterology* 2004; **51**: 887-890

4 **Hyung WJ**, Cheong JH, Kim J, Chen J, Choi SH, Noh SH. Application of minimally invasive treatment for early gastric cancer. *J Surg Oncol* 2004; **85**: 181-185; discussion 186

5 **Ohgaki M,** Toshio T, Akeo H, Yamasaki J, Togawa T. Effect of extensive lymph node dissection on the survival of early gastric cancer. *Hepatogastroenterology* 1999; **46**: 2096-2099

6 **Kubota H,** Tabara H, Kotoh T, Kumar DD, Monden N, Watanabe R, Kohno H, Nagasue N. Prognostic factors and rational approach in the treatment of submucosal cancer of the stomach. *J Surg Res* 1998; **80**: 304-308

7 **Borie F,** Plaisant N, Millat B, Hay JM, Fagniez PL. Appropriate gastric resection with lymph node dissection for early gastric cancer. *Ann Surg Oncol* 2004; **11**: 512-517

8 **Yoshikawa T,** Tsuburaya A, Kobayashi O, Sairenji M, Motohashi H, Noguchi Y. Indications of limited surgery for gastric cancer with submucosal invasion--analysis of 715 cases with special reference to site of the tumor and level 2 lymph nodes. *Hepatogastroenterology* 2003; **50**: 1727-1730

9 **Nitti D,** Marchet A, Mammano E, Ambrosi A, Belluco C, Mencarelli R, Maino M, Marconato G, Farinati F, Lise M.

Extended lymphadenectomy (D2) in patients with early gastric cancer. *Eur J Surg Oncol* 2005; **31**: 875-881
10 **Japanese Gastric Cancer Association.** Japanese Classification of Gastric Carcinoma. 2nd ed. Gastric Cancer 1998; **1**: 10-24
11 **Yoshikawa T,** Tsuburaya A, Kobayashi O, Sairenji M, Motohashi H, Noguchi Y. Is D2 lymph node dissection necessary for early gastric cancer? *Ann Surg Oncol* 2002; **9**: 401-405
12 **Potrc S,** Skalicky M, Ivanecz A. Does endoscopic ultrasound staging already allow individual treatment regimens in gastric cancer. *Wien Klin Wochenschr* 2006; **118** Suppl 2: 48-51
13 **Tsendsuren T,** Jun SM, Mian XH. Usefulness of endoscopic ultrasonography in preoperative TNM staging of gastric cancer. *World J Gastroenterol* 2006; **12**: 43-47
14 **Ganpathi IS,** So JB, Ho KY. Endoscopic ultrasonography for gastric cancer: does it influence treatment? *Surg Endosc* 2006; **20**: 559-562
15 **Chen JQ.** Alternative and Evaluation of minimized Operation or Extended Operation for Gastric Carcinoma. *Zhonghua Weichang Waike Zazhi* 2006, **9**: 8-10
16 **Shimoyama S,** Yasuda H, Mafune K, Kaminishi M. Indications of a minimized scope of lymphadenectomy for submucosal gastric cancer. *Ann Surg Oncol* 2002; **9**: 625-631
17 **Kunisaki C,** Shimada H, Nomura M, Akiyama H. Appropriate lymph node dissection for early gastric cancer based on lymph node metastases. *Surgery* 2001; **129**: 153-157
18 **Shimada S,** Yagi Y, Shiomori K, Honmyo U, Hayashi N, Matsuo A, Marutsuka T, Ogawa M. Characterization of early gastric cancer and proposal of the optimal therapeutic strategy. *Surgery* 2001; **129**: 714-719
19 **Wang FR,** Wang SB. Multivariate Analysis on Risk Factors for Lymph Node Metastasis from Early Gastric Carcinoma. *Zhongguo Zhongliu Linchuang* 2000; **27**: 729-731
20 **Gotoda T,** Yanagisawa A, Sasako M, Ono H, Nakanishi Y, Shimoda T, Kato Y. Incidence of lymph node metastasis from early gastric cancer: estimation with a large number of cases at two large centers. *Gastric Cancer* 2000; **3**: 219-225
21 **Yamao T,** Shirao K, Ono H, Kondo H, Saito D, Yamaguchi H, Sasako M, Sano T, Ochiai A, Yoshida S. Risk factors for lymph node metastasis from intramucosal gastric carcinoma. *Cancer* 1996; **77**: 602-606

S- Editor Zhu LH **L- Editor** Wang XL **E- Editor** Li HY

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5139-5146
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Pretreatment of cromolyn sodium prior to reperfusion attenuates early reperfusion injury after the small intestine ischemia in rats

Zi-Qing Hei, Xiao-Liang Gan, Gang-Jian Luo, Shang-Rong Li, Jun Cai

Zi-Qing Hei, Xiao-Liang Gan, Gang-Jian Luo, Shang-Rong Li, Jun Cai, Department of Anesthesiology, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou 510630, Guangdong Province, China
Supported by The Chinese Traditional Medicine Foundation of Guangdong Province, China, No. 1040051
Correspondence to: Dr. Zi-Qing Hei, Department of Anesthesiology, The Third Affiliated Hospital, Sun Yat-sen University, Guangzhou 510630, Guangdong Province, China. heiziqing0530@hotmail.com
Telephone: +86-20-87580867
Received: June 24, 2007 Revised: July 26, 2007

Key words: Ischemia; Reperfusion injury; Intestinal mucosal mast cells; Histamine; Tumor necrosis factor-α

Hei ZQ, Gan XL, Luo GJ, Li SR, Cai J. Pretreatment of cromolyn sodium prior to reperfusion attenuates early reperfusion injury after the small intestine ischemia in rats. *World J Gastroenterol* 2007; 13(38): 5139-5146

http://www.wjgnet.com/1007-9327/13/5139.asp

Abstract

AIM: To investigate the effects of Cromolyn Sodium (CS) pretreated prior to reperfusion on the activity of intestinal mucosal mast cells (IMMC) and mucous membrane of the small intestine in ischemia-reperfusion (IR) injury of rats.

METHODS: Thirty-two Sprague-Dawley (SD) rats were randomly divided into four groups: sham group (group S), model group (group M), high and low dosage of CS groups, (treated with CS 50 mg/kg or 25 mg/kg, group C1 and C2). Intestinal IR damage was induced by clamping the superior mesenteric artery for 45 min followed by reperfusion for 60 min. CS was intravenouly administrated 15 min before reperfusion. Ultrastructure and counts of IMMC, intestinal structure, the expression of tryptase, levels of malondisldehyde (MDA), TNF-α, histamine and superoxide dismutase (SOD) activity of the small intestine were detected at the end of experiment.

RESULTS: The degranulation of IMMC was seen in group M and was attenuated by CS treatment. Chiu's score of group M was higher than the other groups. CS could attenuate the up-regulation of the Chiu's score, the levels of MDA, TNF-α, and expression of tryptase and the down-regulation of SOD activity and histamine concentration. The Chiu's score and MDA content were negatively correlated, while SOD activity was positively correlated to the histamine concentration respectively in the IR groups.

CONCLUSION: Pretreated of CS prior to reperfusion protects the small intestine mucous from ischemia-reperfusion damage, the mechanism is inhibited IMMC from degranulation.

INTRODUCTION

Mast cell degranulation is an important component of inflammatory tissue responses[1]. Bortolotto proved that ischemia-reperfusion injury was depending upon the presence of mast cells in skeletal muscle[2].

Intestinal mucosal mast cells (IMMCs) are particularly frequent in close proximity to epithelial surfaces where they are strategically located for optimal interaction with the environment and for their putative functions for host defense[3].

Some studies on the role of IMMC in small intestine ischemia-reperfusion injury have been reported[4-7]. Lindestrom and his colleagues[4] found the eosinophils and mast cells in rat ileum gradually increased after intestine ischemia (60 min)/reperfusion (60 min), and reported that the epithelium permeability increased significantly after ischemia-reperfusion. The researches of Kanwar and Schramm[5,6] were focused on mast cells stimulating neutrophil adherence, resupination, recruitment, and the intestinal mucosal injury. Boros[7] reported that mast cell degranulation prior to ischemia may induce a potentially protective mechanism in the small bowel mucosa and decrease ischemia-reperfusion injury in the dog.

Cromolyn Sodium (CS) is a MC membrane stabilizer. MC-stabilization protocols were proved to reduce the leukocyte recruitment[8]. Kimura[9] reported that the intestine ischemia and reperfusion could induce a decrease in the mucosal histamine content, an increase in plasma histamine levels, and an significantly enhance in mucosal permeability. However, MAR-99, another mast cell stabilizer, can prevented these changes by pretreatment prior to ischemia. Kalia[10] found that all ketotifen-pretreated animals (1 mg/kg orally twice daily for 3 d before ischemia)

survived after ischemia-reperfusion and ketotifen could abrogate the leukocyte adherence induced by ischemia-reperfusion within the villus mucosal capillaries and supplying arterioles and largely prevented pulmonary injury.

The above studies proved that IMMC are associated with the small intestine injury after ischemia-rreperfusion, and MC membrane stabilizer pretreatment prior to ischemia can protects against the injury, such as CS and MAR-99. While the studies about the intestinal mucosal injury with CS pretreatment after the small intestine ischemia before reperfusion were few. Oxidative stress is one of the mechanism about the small intestine ischemia-reperfusion injury has been generally acknowledged. We hypothesized that CS have an influence on the oxidative stress during the small intestine ischemia-reperfusion, and the purpose of our present study was to investigate whether CS pretreatment prior to reperfusion could protect against early intestinal mucosal damage induced by ischemia-reperfusion through inhibition of IMMC degranulation or oxidative stress. To test our hypothesis, we showed in a rat IR gut injury model: (1) the ultrastructure and counts of IMMC in the early reperfusion; (2) mucosal damage with CS pretreatment; (3) expression of tryptase in the IMMC; and (4) the levels of malondisldehyde (MDA), TNF-α, histamine and superoxide dismutase (SOD) activity of the small intestine in rats.

MATERIALS AND METHODS

Acute ischemia-reperfusion injury of the intestinal mucosa in rats

Thirty-two healthy Sprague-Dawley rats (200-250 g, provided by Animal Center of Sun Yat-Sen University and approved by the University Animal Study Committee) were randomly divided into four groups each of which contained 8 rats. Laboratory temperature was kept at 25℃-27℃. Surgery was conducted under general anesthesia with intra-peritoneal sodium pentobarbital (45 mg/kg) after they were fasted for 18 h. Tracheotomy was performed for ventilation. The right femoral vein was cannulated for fluid infusion and drugs.The rat abdomen was opened and its superior mesenteric artery (SMA) was found and clamped for 45 min. Then the clamp was released and reperfusion of the splanchnic region was maintained for 60 min (in M group). In control group, SMA was found but not clamped and i.v. saline solution via the right femoral vein at 30th min after the start of experiment (sham group). In another two groups, the same operation was done and CS (50 mg/kg or 25 mg/kg, The dosage and method of CS pretreatment according to Cordeiro *et al*[11] for Cromolyn sodium is poorly absorbed by oral.) was given via right femoral vein 15 min before the opening of the clamp (C1 and C2 groups).

Preparation of specimens and measurements

After ischemia-reperfusion, the rats were killed and paunched rapidly. A segment of 0.5-1.0 cm intestine was cut from 5 cm to terminal ileum and fixed in 4% formaldehyde polymerisatum, then embedded in paraffin for section. Another segment of small intestine was washed with frozen saline and dried with suction paper and at -70℃.

The segment of small intestine was stained with hematoxylin-eosin. The damages of intestinal mucosa were evaluated by two different pathologist according to the criteria of Chiu's method[12]. Criteria of Chiu grading system consists from 5 subdivisions according to the changes of villus and gland of intestinal mucosa: grade 0, normal mucosa; grade 1, development of subepithelial Gruenhagen's space at the tip of villus; grade 2, extension of the space with moderate epithelial lifting; grade 3, massive epithelial lifting with a few denuded villi; grade 4, denuded villi with exposed capillaries; and grade 5, disintegration of the lamina propria, ulceration and hemorrhage.

Transmission electron microscopy

Intestines were immersed and fixed in 2.5% glutaraldehyde overnight at 4℃ and washed three times in PBS. Then they were postfixed in aqueous 1% OsO_4 and 1% $K_3Fe(CN)_6$ for 1 h. After three times of PBS washes, the tissue was dehydrated through a graded series of 30% to 100% ethanol and 100% propylene oxide and then infiltrated in 1:1 mixture of propylene oxide and Polybed 812 epoxy resin for 1 h. The infiltration solution was changed to 100% resin. After 24 h of infiltration, the tissue was embedded in molds and cured at 37℃ overnight, followed by additional hardening at 65℃ for 2 d. Ultrathin (70 nm) sections were collected on 200-mesh copper grids and stained with 2% uranyl acetate in 50% methanol for 10 min, followed by 1% lead citrate for 7 min. Sections were photographed using a Hitachi H-600 transmission electron microscope (TOSHIBA, Japan) at 80 kV onto electron microscope film.

Detection of concentration of protein in intestine

Intestinal tissues were homogenized with normal saline. Intestinal protein quantitation was by the Bradford method[13] with a BSA standard using kits were provided by Shenerg Biocolor BioScience & Technolgy Company, Shanghai, China.

Detection of content of MDA in the intestine

Intestinal tissues were homogenized with normal saline. MDA content was determined by the TBA method (Jiancheng Bioengineering Ltd, Nanjing, China). Homogenate (0.1 mL) was taken to detect MDA content. Briefly, 0.1 mL 8.1% SDS, 0.8 mL acetic acid buffer, 0.8 mL 0.8% TBA and 0.2 mL distilled water were added into the sample tubes and one standard tube (containing 0.1 mL tetraethoxypropane). All the tubes were then incubated at 100℃ for 1 h. After cooled at -20℃ for 5 min, 2 mL of n-butyl alcohol was added into the sample, which was then vibrated for 1 minute and centrifuged for 10 min at 3000 r/min. The supernatant of the samples were assayed to detect absorbance at 532 nm; and the results were expressed as nmol/mL. The content of MDA in intestine was calculated as millimicromole per milligram of protein.

Detection of activity of SOD in the intestine

Intestinal tissues were made into a homogenate with normal saline, frozen at -20℃ for 5 min and centrifuged for 15 min at 4000 r/min. Supernatants were transferred into fresh tubes for evaluation of SOD activity. SOD activity was evaluated with an SOD detection kit according to the

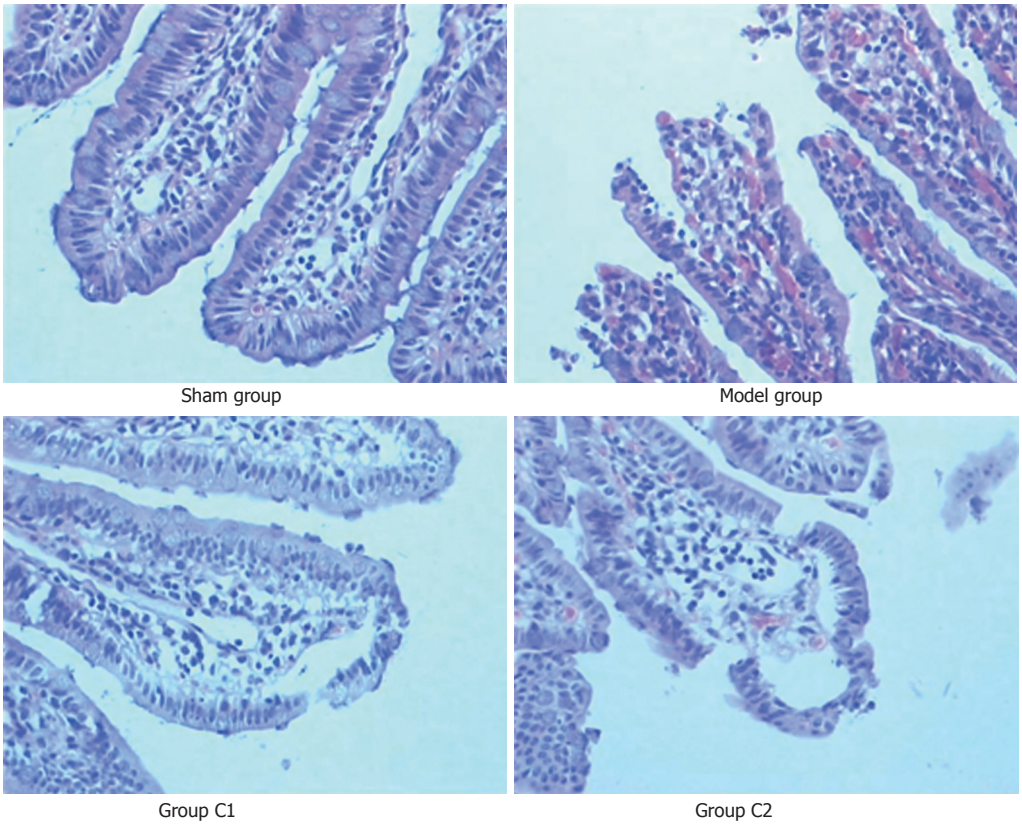


Figure 1 Microscopic appearance after hematoxylin and eosin staining (× 200). In the model group, there are multiple erosions and bleeding in mucosal epithelial layer; the villus and glands were normal and no inflammatory cell infiltration was observed in mucosal epithelial layer in sham group; these mucosal changes are ameliorated by treatment with Cromolyn Sodium (group C1 and group C2).

manufacturer's instructions (Jiancheng Bioengineering Ltd, Nanjing, China). Results were expressed as nmol/mL. The activity of SOD in the intestine was calculated as U per milligram of protein.

Detection of the concentration of TNF-α in the intestine

Intestinal tissues were made into a homogenate with normal saline, frozen at -20℃ for 5 min and centrifuged for 15 min at 4000 r/min. Supernatants were transferred into fresh tubes for evaluation of concentration of TNF-α (Biosource, USA) using a commercially available ELISA kit in accordance with the manufacturer's instructions, results were expressed as pg/mL. The concentration of TNF-α in the intestine was calculated as picogram per milligram of protein.

Detection of the concentration of histamine in the intestine

Intestinal tissues were made into a homogenate with normal saline, frozen at -20℃ for 5 min and centrifuged for 15 min at 4000 r/min. Supernatants were transferred into fresh tubes for evaluation of concentration of histamine (RapidBio Lab, USA) using a commercially available ELISA kit in accordance with the manufacturer's instructions, results were expressed as ng/mL. The concentration of histamine in the intestine was calculated as nanogram per milligram of protein.

Immunohistochemical detection of tryptase in intestine

Five μm thick sections were prepared from paraffin-embedded tissue. After deparaffinization, endogenous peroxidase was quenched with 3% H_2O_2 in deionised water for 10 min. Nonspecific binding sites were blocked by incubating the sections in 10% normal rabbit serum for 1 h. The sections were then incubated with polyclonal rat anti-mast cell tryptase (dilution 1: 50) for 30 min at 37℃, followed by incubation with biotinylated goat-anti-rat IgG at room temperature for 10-15 min. After 3 × 5 min PBS rinses, the horseradish-peroxidase-conjugated streptavidin solution was added and incubated at room temperature for 10-15 min. The antibody binding sites were visualized by incubation with a diaminobenzidine-H_2O_2 solution. The sections incubated with PBS instead of the primary antibody were used as negative controls. Brown-yellow granules in the cytoplasm were recognized as positive staining for tryptase. We calculated the tryptase positive mast cells and their intensity in 5 representative areas at × 400 magnification by Image-Pro Plus 5.0 (USA).

Statistical analysis

Data were expressed as mean ± SD and analysis of variance was performed using SPSS 11.0 software. One-way analysis of variance was used for multiple comparison, least significant difference test (LSD-t) was used for intra-group comparison or Tamhane's T2 test was used if equal variances was not assumed. Pearson analysis was used for the correlation in the ischemia and reperfusion groups. Differences were considered significant when P was < 0.05.

RESULTS

Changes of intestinal mucosa under light microscope

The villus and glands were normal and no inflammatory cell infiltration was observed in mucosal epithelial layer in sham group. Multiple erosions and bleeding were observed in model group. Light edema of mucosa villus and infiltration of few necrotic epithelial inflammatory cells neutrophil leukomonocyte were found in mucosa epithelial layer in C2 and C1 groups (Figure 1).

Table 1 Changes of IMMC counts, expression of tryptase, and Chiu's score in small intestine in various groups (mean ± SD)

Group	*n*	IMMC (*n*/field)	Expression of tryptase	Chiu's score
S	8	10 ± 2	126 ± 4	0.4 ± 0.5
M	8	25 ± 8[b]	173 ± 4[b]	4.9 ± 0.4[b]
C1	8	15 ± 2[a]	138 ± 3[b,d]	2.4 ± 0.5[b,d]
C2	8	17 ± 2[a]	155 ± 8[b,d,f]	2.9 ± 0.4[b,d,e]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* Group S; [d]$P < 0.01$ *vs* Group M; [e]$P < 0.05$, [f]$P < 0.01$ *vs* Group C1.

Chiu's score of small intestinal structure

The Chiu's score in sham group was the lowest, while in the model group it was the highest in the four groups ($P < 0.05$). The Chiu's score in C1 group was significantly lower than in C2 group after treated with CS ($P < 0.05$) (Table 1).

Changes of ultrastructure of small intestinal

The ultrastructure of small intestinal was normal in group S. There was seen the karyopyknosis of epithelial cell of small intestine in group M, the nuclear membrane was more irregularity, and the swelling microvillus became shorter and thicker, most of the microvillus were shedding. The nucleus of epithelial cell of small intestine in group C1 and C2 was deflated, the nuclear membrane was irregularity, and the light swelling microvillus became shorter (Figure 2).

Changes of ultrastructure of IMMC

The ultrastructure of IMMC was normal in sham group. There were abundant vacuolus with a reduction granulation in their endochylema in model group. There were few swollen granules with a reduction in IMMC homogeneity in C1 and C2 group (Figure 3).

Changes of MDA in small intestine

The content of MDA in intestine of model group was the highest in all experimental groups, it decreased significantly compared with the model group after treated with CS ($P < 0.05$) and there was no significant difference compared with the sham group ($P > 0.05$) (Table 2).

Changes of activity of SOD in small intestine

The activity of intestinal SOD decreased significantly in ischemia-reperfusion injury groups compared with the sham group ($P < 0.05$), treated with CS it increased significantly compared with the model group ($P < 0.05$), and there was no significant difference between group C1 and C2 ($P > 0.05$) (Table 2).

Changes of TNF-α in small intestine

The concentration of TNF-α of intestine in model group rats was higher than the other three groups ($P < 0.05$). There were no significant difference in sham group, C1 group and C2 group ($P > 0.05$) (Table 2). There was a positive correlation between the Chiu's score and the concentration of TNF-α in the ischemia and reperfusion groups ($r = 0.734$, $P < 0.05$).

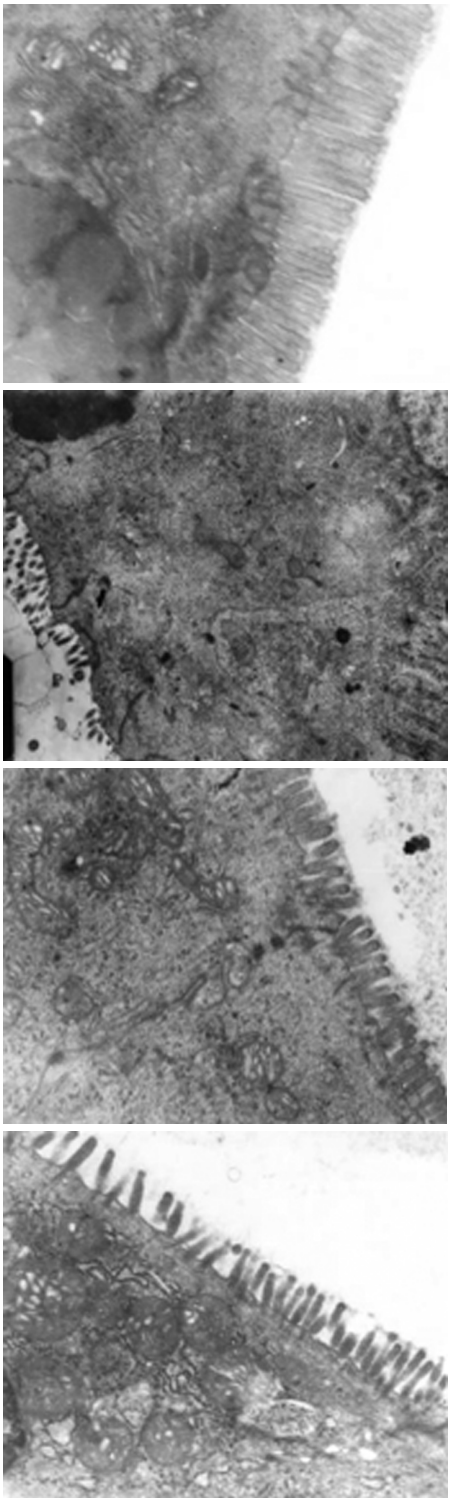


Figure 2 Changes of ultrastructure of small intestinal in each group (× 10000). The ultrastructure of small intestinal was normal in group S. There were seen the karyopyknosis of epithelial cell of small intestine in group M, and the nuclear membrane was more irregularity, and the swelling microvillus became shorter and thicker, most of the microvillus were shedding. The nucleus of epithelial cell of small intestine in group C1 and C2 was deflated, the nuclear membrane was irregularity, and the microvillus became shorter and light swelling.

Changes of histamine in small intestine

The histamine concentration of intestine in the model and C2 groups decreased significantly compared with the sham group ($P < 0.05$), it increased significantly after pretreated with CS compared with the model group ($P < 0.05$). There

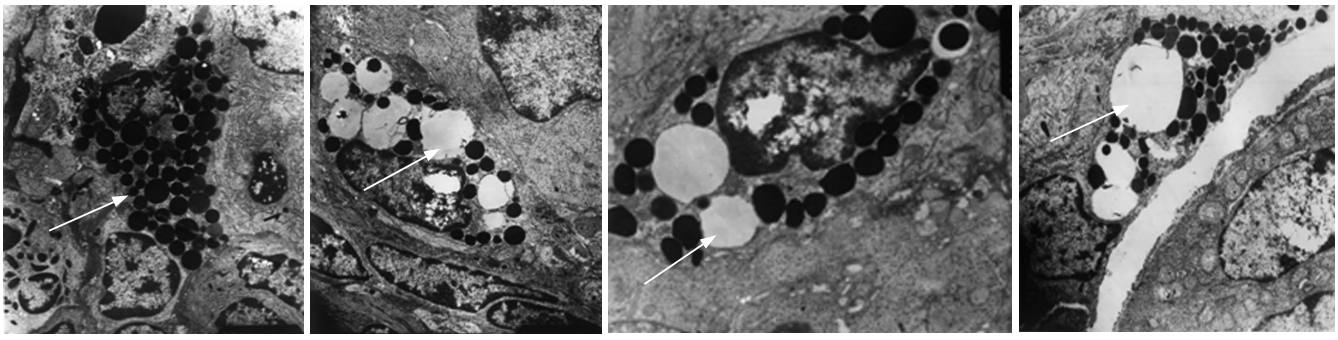


Figure 3 Ultrastructure of intestinal mucosal mast cells of rats in each group. There are abundant vacuolus with a reduction of granulation in their endochylema in model group; there are filled with granulation endochylema and there is no vacuolus in their endochylema in sham group, these changes of ultrastructure are ameliorated by treatment with Cromolyn Sodium (group C1 and C2).

Table 2 Changes of TNF-α, MDA content, SOD activity and histamine concentration in small intestine in various groups (mean ± SD)

Group	*n*	TNF-α (pg/mg protein)	MDA (nmol/mg protein)	SOD (U/mg protein)	Histamine (ng/mg protein)
S	8	3.7 ± 0.4	0.44 ± 0.09	179.2 ± 15.3	5.7 ± 0.5
M	8	4.7 ± 0.4[b]	0.66 ± 0.07[b]	130.6 ± 10.6[b]	4.2 ± 0.4[b]
C1	8	3.8 ± 0.4[d]	0.45 ± 0.06[d]	147.9 ± 12.4[b,c]	5.3 ± 0.6[d]
C2	8	3.9 ± 0.4[d]	0.47 ± 0.07[d]	145.3 ± 15.7[b,c]	4.8 ± 0.6[a,c]

[a]$P < 0.05$, [b]$P < 0.01$ *vs* Group S; [c]$P < 0.05$, [d]$P < 0.01$ *vs* Group M.

were no significant difference between C1 and C2 groups ($P > 0.05$) (Table 2). The Chiu's score and MDA content were negatively correlated to the histamine concentration respectively ($r = -0.676$, $P < 0.05$ or $r = -0.452$, $P < 0.05$), while the SOD activity was positively correlated to the concentration of histamine in the ischemia and reperfusion groups ($r = 0.579$, $P < 0.05$).

Counts and expression of trypatse of IMMC

Expression of tryptase in sham group was the lowest, while in the model group it was the highest in the four groups ($P < 0.05$), and the expression of tryptase in C1 group was significantly lower than in C2 group ($P < 0.05$). The number of IMMC increased significantly in ischemia-reperfusion injury groups compared with the sham group ($P < 0.05$), no difference was compared among the three groups ($P > 0.05$) (Table 1, Figure 4).

DISCUSSION

IMMC are located in close proximity to submucosal collecting venules, which are primary targets of leukocyte-endothelial interactions during ischemia-reperfusion injure. IMMC are particularly frequent in close proximity to epithelial surfaces where they are strategically located for optimal interaction with the environment and for their putative functions for host defense. They sense the foreign material invading the mucosa in an appropriate inflammatory response, and were considered as one of components of the fourth level of mucosal defense[14]. Acute inflammation could lead to increase of IMMC counts and release of a multi-faceted spectrum of proinflammatory mediators by IMMC such as cytokines and chemokines, and MC have the capacity to coordinate trafficking of leukocytes[15]. Boros[16] proved that intestinal ischemia induced the release of a variety of IMMC-derived inflammatory compounds and resulted in a spectrum of injury ranging from reversible permeability changes to structural mucosal damage.

The ischemia time of small intestine rats' model is from 30 min to 60 min[17-19], here we used the median time (45 min). Cizova reported that the concentration of thiobarbituric acid reactive substances was increased at the end of the ischemia lasting from 30 to 90 min[20], and CS plasma life *in vivo* is very short. Thus the reperfusion time in our study was watched in 60 min, it was the early reperfusion according to Hamar *et al*[21]. All of previous studies were focused on the MC membrane stabilizer pretreatment prior to ischemia, and had proved that IMMC were associated with the damage to intestinal mucosal after the small intestine ischemia-reperfusion. While the main purpose of our study was to see whether pretreatment with CS prior to reperfusion also have the protective effects during early reperfusion after the small intestine ischemia.

Tryptase is one of the specificity markers of IMMC[22]. We counted the IMMC counts through the expression of tryptase using immunohistochemical methods which is more accuracy than oluidine blue staining. Our study found that the expression of tryptase and IMMC counts increased significantly in 60 min reperfusion injury in model group. There were abundant vacuolus in IMMCs in the model group after they were degranulated by electron microscope. IMMC is the main source of histamine in intestine. The levels of intestinal histamine includes the concentration of histamine intra- and extro-IMMC. The level of intestinal histamine is mainly represent of the concentration of histamine intra-IMMC as the extracellular

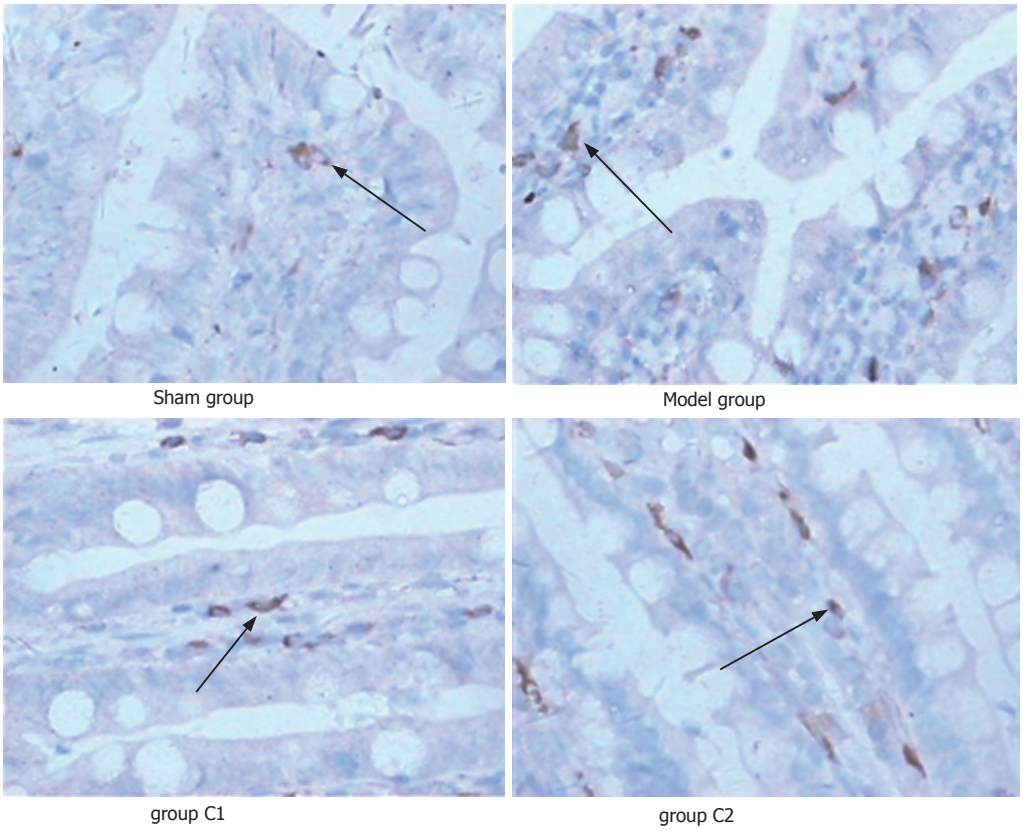


Figure 4 Immunohistochemical detection of tryptase in small intestinal of rats in each group (×400). Expression of tryptase and number of IMMC are increased in the model group; these changes were ameliorated by treatment with Cromolyn Sodium (group C1 and C2).

histamine released from IMMC in the gastrointestinal tract is rapidly cleared and degraded[23] and the more IMMC degranulate, the lower concentration of histamine is found in intestine[24]. Our study found the histamine level decreased significantly in model group while IMMC counts increased, suggesting that IMMC may degranulate and release histamine in 60 min reperfusion.

Histamine has many pathophysiological roles, and it is an important messenger in the gut[25]. Akerstrom *et al*[26] reported that anti-histaminergic pretreatment could decrease the trauma-induced leakage of albumin by mechanisms which may involve readjustments of pressures and flows in capillaries as well as a prevention of histamine effects on capillary permeability on a model of mechanical intra-abdominal trauma in rats. Our results found that the intestinal histamine concentration decreased after early ischemic-reperfusion, and there was a negative corrletion between the small intestinal Chiu's score and the level of histamine in the intestine. This result suggested that histamine took part in the ischemia-reperfusion intestinal mucosal damage.

TNF-α is an inflammatory cytokine that may be an important mediator in the development of reperfusion-induced tissue injury and lethality[27]. Grewal[28] demonstrated that treatment of rats with anti-TNF antiboies could prevent neutrophil influx, tissue injury. Our study found that the intestinal TNF-α concentration increased after ischemic-reperfusion; and there was a positive correlation between the small intestinal Chiu's score and the level of TNF-α in the intestine. This result suggested that TNF-α also took part in the ischemia-reperfusion intestinal mucosal damage. Althrough the most intestinal TNF-α is considered by some one from the mast cells[29], it has been proved that many sorts of cells also release the TNF-α besides the mast cells. We believe the increase of the TNF-α is contributed by many factors.

CS is a stabilizing agent of mast cell which prevents histamine and TNF-α released from IMMC[30]. Szabo *et al*[31] reported that 30 min segmental ischemia and 120 min reperfusion induced significant tissue injury, elevated the segmental vascular resistance, and decreased intramucosal pH (pHi), and CS pretreatments prior to ischemia significantly inhibited the permeability changes, but did not influence the pHi and morphological alterations induced by ischemia-reperfusion, they conclude that intestinal mast cells and mast cell-induced reactions contribute to the mucosal permeability alterations during reperfusion, but play only a minor role in ischemia-reperfusion-induced structural injury. Pretreatment with CS protecting against degranulation, caused a significant impairment of plasma exudation at 30 min of inflammation corresponding to a significantly decreased level of histamine, one of the most potent vasoactive factors released from activated mast cells[32].

The results in our study showed that the injury of small intestinal villus and microvillus was alleviated after CS pretreatment prior to reperfusion and that the ultrastructure of IMMC was basically normal. The expression of tryptase and TNF-α concentration were also alleviated by CS pretreatment prior to reperfusion, and the concentration of histamine in intestine was increased compared with the model group after CS pretreatment prior to reperfusion. The results indicated that CS decreased ischemia-reperfusion injury by prevention of IMMC degranulation, thus it decreased the release of histamine and TNF-α. This protection may be dose-dependent as high dose of CS with

more powerful effect.

There were many reports about intestinal ischemia and reperfusion resulted in the increase of MDA and decrease of SOD activity, toxic-free oxygen radicals are produced in the ischemic tissue[33,34]. Our study also demonstrated that ischemia-reperfusion injury elevated the oxygen radicals and lipid radicals. Frossi[35] reproted that oxidative stress could induce a pro-type 2 inflammatory response and degranulation of mast cells. Fukuishi[36] found the compound 48/80, a typical histamine liberator elicited superoxide anion generation in mast cells in a dose-dependent fashion. These studies indicated that degranulation of mast cells was able to induce oxidative stress injury and oxygen radicals could make mast cells to degranulate. In this study we found there were correlations among the MDA, SOD activity and the concentration of histamine, the other findings of our study were that MDA content increased and SOD activity decreased remarkably in the model group, while pretreatment by cromolyn sodium prior to reperfusion could attenuate the up-regulation of MDA content and the down-regulation of SOD activity. The results were indicating that IMMC degranulation and oxidative stress can affect each other, and the less IMMC degranulation can make less oxidative stress. Future studies are need to focus on the relationships *in vitro*.

In conclusion, pretreatment of Cromolyn Sodium prior to reperfusion could attenuate early reperfusion injury after the small intestine ischemia in rats. The mechanisms includes: inhibitied IMMC from degranulation, decreased the release of histamine and TNF-α from IMMC, and decreased oxidative stress.

COMMENTS

Background

Intestinal mucosal mast cells (IMMCs) is associated with the mucosal damage. The aim of this study was to investigate the effects of Cromolyn Sodium (CS) pretreated prior to reperfusion on the activity of IMMC and mucous membrane of the small intestine in ischemia-reperfusion (IR) injury of rats.

Research frontiers

Previous studies proved that IMMC are associated with the small intestine injury after ischemia-reperfusion, and MC membrane stabilizer pretreatment prior to ischemia can protects against the injury, such as CS and MAR-99.

Innovations and breakthroughs

While the studies about the intestinal mucosal injury with CS pretreatment after the small intestine ischemia before reperfusion were few. Oxidative stress is one of the mechanism about the small intestine ischemia-reperfusion injury has been generally acknowledged. We hypothesized that CS have an influence on the oxidative stress during the small intestine ischemia-reperfusion, and the purpose of our present study was to to investigate whether CS pretreatment prior to reperfusion could protect against early intestinal mucosal damage induced by ischemia-reperfusion through inhibition of IMMC degranulation or oxidative stress.

Applications

Pretreated of CS prior to reperfusion protects the small intestine mucous from ischemia-reperfusion damage, the mechanism is inhibited IMMC from degranulation.

Terminology

Restoration of blood supply to tissue which is ischemic due to decrease in normal blood supply. The decrease may result from any source including atherosclerotic obstruction, narrowing of the artery, or surgical clamping. It is primarily a procedure for treating infarction or other ischemia, by enabling viable ischemic tissue to recover, thus limiting further necrosis. However, it is thought that reperfusion can itself further damage the ischemic tissue, causing REPERFUSION INJURY.

Peer review

This paper reports an experimental study very well designed and performed and very elegant results and discussion. The final conclusions are nicely shown. Their english is of good quality. The purpose of this paper was to determine whether cromolyn sodium reduces or prevents injury of the small intestine of rats following ischemia-reperfusion. To this end the authors perform a number of biochemical measurements (MDA, TNFalpha, histamine, SOD) as well as ultrastructural studies of mast cells and microscopical investigations of the small intestine.

REFERENCES

1 **Boros M**, Takaichi S, Masuda J, Newlands GF, Hatanaka K. Response of mucosal mast cells to intestinal ischemia-reperfusion injury in the rat. *Shock* 1995; **3**: 125-131
2 **Bortolotto SK**, Morrison WA, Messina A. The role of mast cells and fibre type in ischaemia reperfusion injury of murine skeletal muscles. *J Inflamm* (Lond) 2004; **1**: 2
3 **Kanwar S**, Kubes P. Mast cells contribute to ischemia-reperfusion-induced granulocyte infiltration and intestinal dysfunction. *Am J Physiol* 1994; **267**: G316-G321
4 **Lindeström LM**, Ekblad E. Structural and neuronal changes in rat ileum after ischemia with reperfusion. *Dig Dis Sci* 2004; **49**: 1212-1222
5 **Kanwar S**, Wallace JL, Befus D, Kubes P. Nitric oxide synthesis inhibition increases epithelial permeability via mast cells. *Am J Physiol* 1994; **266**: G222-G229
6 **Schramm R**, Thorlacius H. Neutrophil recruitment in mast cell-dependent inflammation: inhibitory mechanisms of glucocorticoids. *Inflamm Res* 2004; **53**: 644-652
7 **Boros M**, Kaszaki J, Ordögh B, Nagy S. Mast cell degranulation prior to ischemia decreases ischemia-reperfusion injury in the canine small intestine. *Inflamm Res* 1999; **48**: 193-198
8 **Holian A**, Hamilton R, Scheule RK. Mechanistic aspects of cromolyn sodium action on the alveolar macrophage: inhibition of stimulation by soluble agonists. *Agents Actions* 1991; **33**: 318-325
9 **Kimura T**, Fujiyama Y, Sasaki M, Andoh A, Fukuda M, Nakajima S, Bamba T. The role of mucosal mast cell degranulation and free-radical generation in intestinal ischaemia-reperfusion injury in rats. *Eur J Gastroenterol Hepatol* 1998; **10**: 659-666
10 **Kalia N**, Brown NJ, Wood RF, Pockley AG. Ketotifen abrogates local and systemic consequences of rat intestinal ischemia-reperfusion injury. *J Gastroenterol Hepatol* 2005; 20: 1032-1038
11 **Cordeiro PG**, Lee JJ, Mastorakos D, Hu QY, Pinto JT, Santamaria E. Prevention of ischemia-reperfusion injury in a rat skin flap model: the role of mast cells, cromolyn sodium, and histamine receptor blockade. *Plast Reconstr Surg* 2000; **105**: 654-659
12 **Chiu CJ**, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch Surg* 1970; **101**: 478-483
13 **Bradford MM**. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; **72**: 248-254
14 **Penissi AB**, Rudolph MI, Piezzi RS. Role of mast cells in gastrointestinal mucosal defense. *Biocell* 2003; **27**: 163-172
15 **Galli SJ,** Maurer M, Lantz CS. Mast cells as sentinels of innate immunity. *Curr Opin Immunol* 1999; **11**: 53-59
16 **Boros M**. Microcirculatory dysfunction during intestinal ischemia-reperfusion. *Acta Physiol Hung* 2003; **90**: 263-279
17 **Ito K**, Ozasa H, Horikawa S. Edaravone protects against lung injury induced by intestinal ischemia/reperfusion in rat. *Free Radic Biol Med* 2005; **38**: 369-374
18 **Andoh A**, Fujiyama Y, Araki Y, Kimura T, Tsujikawa T, Bamba

T. Role of complement activation and mast cell degranulation in the pathogenesis of rapid intestinal ischemia/reperfusion injury in rats. *Digestion* 2001; **63** Suppl 1: 103-107

19 **Tomatsuri N**, Yoshida N, Takagi T, Katada K, Isozaki Y, Imamoto E, Uchiyama K, Kokura S, Ichikawa H, Naito Y, Okanoue T, Yoshikawa T. Edaravone, a newly developed radical scavenger, protects against ischemia-reperfusion injury of the small intestine in rats. *Int J Mol Med* 2004; **13**: 105-109

20 **Cízová H**, Lojek A, Kubala L, Cíz M. The effect of intestinal ischemia duration on changes in plasma antioxidant defense status in rats. *Physiol Res* 2004; **53**: 523-531

21 **Hamar J**, Rácz I, Cíz M, Lojek A, Pállinger E, Furész J. Time course of leukocyte response and free radical release in an early reperfusion injury of the superior mesenteric artery. *Physiol Res* 2003; **52**: 417-423

22 **Marone G**, Triggiani M, Genovese A, De Paulis A. Role of human mast cells and basophils in bronchial asthma. *Adv Immunol* 2005; **88**: 97-160

23 **Enerback L**. The mast cell system. In: Lane DA, Lindahl U, editors. Heparin. Landon: Edward Arnold, 1989: 97-113

24 **Boros M**, Ordögh B, Kaszaki J, Nagy S. The role of mast cell degranulation in ischaemia-reperfusion-induced mucosal injury in the small intestine. *Ann Acad Med Singapore* 1999; **28**: 79-84

25 **Rangachari PK**. Histamine: mercurial messenger in the gut. *Am J Physiol* 1992; **262**: G1-G13

26 **Akerström G**, Lisander B. Antihistaminergic pretreatment prevents tissue extravasation of albumin from intra-abdominal trauma in rats. *Acta Anaesthesiol Scand* 1994; **38**: 569-574

27 **Souza DG**, Soares AC, Pinho V, Torloni H, Reis LF, Teixeira MM, Dias AA. Increased mortality and inflammation in tumor necrosis factor-stimulated gene-14 transgenic mice after ischemia and reperfusion injury. *Am J Pathol* 2002; **160**: 1755-1765

28 **Grewal HP**, Mohey el Din A, Gaber L, Kotb M, Gaber AO. Amelioration of the physiologic and biochemical changes of acute pancreatitis using an anti-TNF-alpha polyclonal antibody. *Am J Surg* 1994; **167**: 214-218; discussion 218-219

29 **Frangogiannis NG**, Smith CW, Entman ML. The inflammatory response in myocardial infarction. *Cardiovasc Res* 2002; **53**: 31-47

30 **Shin HY**, Kim JS, An NH, Park RK, Kim HM. Effect of disodium cromoglycate on mast cell-mediated immediate-type allergic reactions. *Life Sci* 2004; **74**: 2877-2887

31 **Szabó A**, Boros M, Kaszaki J, Nagy S. The role of mast cells in mucosal permeability changes during ischemia-reperfusion injury of the small intestine. *Shock* 1997; **8**: 284-291

32 **Dib M**, Zhao X, Wang X, Andersson R. Mast cells contribute to early pancreatitis-induced systemic endothelial barrier dysfunction. *Pancreatology* 2002; **2**: 396-401

33 **Yoshimaru T**, Suzuki Y, Inoue T, Niide O, Ra C. Silver activates mast cells through reactive oxygen species production and a thiol-sensitive store-independent Ca2+ influx. *Free Radic Biol Med* 2006; **40**: 1949-1959

34 **Olguner C**, Koca U, Kar A, Karci A, Işlekel H, Canyilmaz M, Mavioğlu O, Kizildağ S, Unlü G, Elar Z. Ischemic preconditioning attenuates the lipid peroxidation and remote lung injury in the rat model of unilateral lower limb ischemia reperfusion. *Acta Anaesthesiol Scand* 2006; **50**: 150-155

35 **Frossi B**, De Carli M, Daniel KC, Rivera J, Pucillo C. Oxidative stress stimulates IL-4 and IL-6 production in mast cells by an APE/Ref-1-dependent pathway. *Eur J Immunol* 2003; **33**: 2168-2177

36 **Fukuishi N**, Sakaguchi M, Matsuura S, Nakagawa C, Akagi R, Akagi M. The mechanisms of compound 48/80-induced superoxide generation mediated by A-kinase in rat peritoneal mast cells. *Biochem Mol Med* 1997; **61**: 107-113

S- Editor Liu Y **L- Editor** Li M **E- Editor** Li JL

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 28; 13(38): 5147-5150
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Wilson disease: Identification of two novel mutations and clinical correlation in Eastern Chinese patients

Sheng Ye, Liang Gong, Quan-Xiang Shui, Lin-Fu Zhou

Sheng Ye, Quan-Xiang Shui, Department of Pediatrics, Child Hospital, Zhejiang University, Hangzhou 310005, Zhejiang Province, China
Liang Gong, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310005, Zhejiang Province, China
Lin-Fu Zhou, Institute of Medical Biotechnology, Zhejiang University, Hangzhou 310005, Zhejiang Province, China
Correspondence to: Lin-Fu Zhou, Institute of Medical Biotechnology, Faculty of Basic Medicine, School of Medicine, Zhejiang University, Hangzhou 310005, Zhejiang Province, China. 239zlf@zju.edu.cn
Telephone: +86-571-85937583 Fax: +86-571-88208238
Received: June 4, 2007 Revised: July 23, 2007

Abstract

AIM: To study mutations in the P-type ATPase (ATP7B) gene responsible for Wilson disease (WD) in the Eastern Chinese population, and the possible correlation of specific mutations with clinical characteristics.

METHODS: Mutations of the ATP7B gene were sought by means of direct sequencing in 50 Eastern Chinese WD patients of Han ethnic origin.

RESULTS: Two novel mutations, Asp96Gly and Asp196Glu, were first identified. We also compared the characterization of mutations in ATP7B with the clinical findings, and a significant correlation with hepatic manifestations between patients carrying the Arg778Leu mutation and those without was found.

CONCLUSION: Gene sequencing analysis was shown to have a high detection rate and accuracy. It may become the first priority in screening of WD patients.

Key words: Wilson disease; ATP7B gene; Mutations; Polymorphisms

Ye S, Gong L, Shui QX, Zhou LF. Wilson disease: Identification of two novel mutations and clinical correlation in Eastern Chinese patients. *World J Gastroenterol* 2007; 13(38): 5147-5150

http://www.wjgnet.com/1007-9327/13/5147.asp

INTRODUCTION

Wilson disease (WD, hepatolenticular degeneration) is an autosomal recessive disorder with an incidence of 1 in 35 000 to 100 000 live births[1-3]. It is characterized by pathological copper accumulation in different tissues, especially in the liver and brain. As a result of defective putative copper-transporting ATPase in the liver, copper remains in the liver and causes hepatic dysfunction. The clinical presentation of WD consists of hypoceruloplasminemia, presence of Kayser-Fleischer (KF) rings, and hepatic, psychiatric and/or neurological disturbance[4]. Treatment of WD has progressed from chelation therapy using D-penicillamine and trientine to the more recent use of zinc, and finally to liver transplantation for fulminant presentation[5]. Timely diagnosis and treatment may protect patients from severe organ damage. The diagnosis of WD is determined by clinical presentation and laboratory testing for KF rings, hepatic injury and low serum ceruloplasmin. However, some asymptomatic patients do not receive effective treatment before irreversible injury is present. Genetic diagnosis may detect presymptomatic patients, in whom initiation of prophylactic therapy can effectively prevent the otherwise inevitable hepatic and neurological injury[6,7].

Mutations in the P-type ATPase (ATP7B, MIM#277900) gene are responsible for WD. The ATP7B gene, which was identified in 1993, is located on chromosome 13q14.3, which spans a genomic region of ~80 kb. It comprises 21 exons and encodes for a P-type copper-transporting ATPase[8-10]. Direct mutation analysis has been performed in many WD patients and > 200 different ATP7B mutations have been detected[11-13]. H1069Q is the most common type of mutation, with an allelic frequency of 10%-40% in European patients[14-16]. However, Arg778Leu in exon 8 is the most frequently observed mutational type in Chinese, Japanese and Korean patients[17-22]. It has been reported that the Arg778Leu mutation may be correlated with hepatic manifestations in Chinese patients[12]. However, the mutations in Chinese patients with WD, and the possible correlation of specific mutations with clinical characteristics, have not been addressed.

In the present study, mutations of the ATP7B gene were sought by means of direct sequencing in 50 Eastern Chinese WD patients of Han ethnic origin, who comprise 99% of the population of mainland China. We also

compared the characterization of mutations in ATP7B with the clinical findings, and report the preliminary results of the genotype/phenotype correlation.

MATERIALS AND METHODS

Subjects

A total of 50 unrelated Han ethnic subjects (24 female, 26 male) in mainland Eastern China were diagnosed as being affected by WD. They were mainly from Zheijang, Anhui and Fujian Provinces. They were recruited when they came to the hospital for seeking assistance. The diagnosis was based on the presence of hepatic disturbance, typical neurological symptoms, KF rings, biochemical tests (low serum concentrations of ceruloplasmin and copper, and high urinary and hepatic copper content). Each of these patients had a score of at least 3 according to a scoring system based on clinical and biochemical parameters[23]. DNA samples from 100 healthy Chinese individuals (50 female, 50 male) were screened to determine whether the missense/splicing mutations identified in this study were present in the normal population. The healthy subjects were mainly students in our teaching hospitals. Informed consent was obtained from all patients or their parents before inclusion in the study.

PCR and DNA sequencing

Anticoagulated blood samples were obtained from the WD patients. Genomic DNA was isolated from peripheral blood lymphocytes by a DNA extractor kit (Qiagen, Germany). All 21 exons of ATP7B were amplified by polymerase chain reaction (PCR). PCR was performed in a 50-μL reaction volume containing 800 ng genomic DNA, 2.5 U Taq polymerase (Takara, Japan), 12.5 pmol each primer, 3 mmol/L $MgCl_2$, 200 μmol/L each dNTP, and 1 × PCR Buffer (Takara, Japan). The PCR reaction conditions were as follows: 15 min initial activation step at 94℃; 35 cycles of denaturation at 94℃ for 30 s, annealing at 55℃ for 30 s and extension at 72℃ for 120 s; and a final extension at 72℃ for 10 min. PCR products were visualized on 2% agarose gel containing ethidium bromide, and subsequently purified using QIAEXⅡ (Qiagen, Germany). Direct sequencing was performed using an ABI 377 fluorescent sequencer (Applied Biosystems, Foster City, CA, USA).

Analysis of genotype–phenotype correlations

We analyzed the correlation between Arg778Leu genotype and WD phenotype, including age of onset and initial symptoms. Data were analyzed using SPSS 13.0. Age of onset of symptoms was compared using Student's *t* test. Categorical variables were compared between groups by the χ^2 test or Fisher's exact test. Differences were considered to be significant at the $P < 0.05$ level.

RESULTS

Mutation analysis

DNA from the 50 patients with WD was screened for mutations. Exons 1-21 were PCR-amplified and the products were sequenced bidirectionally. Mutations in ATP7B were found in at least one of the alleles in all the patients with WD, distributed throughout the gene. In eight patients, only one allelic change was detected, which suggests that the other one might have been located in the introns or regulatory regions. We identified five different mutations (Table 1). Two different mutations found in exons 8 and 13 account for about 77% of all 100 WD alleles studied, thereby indicating that these exons are important regions for detecting mutations in Eastern Chinese patients with WD. The most frequent mutation, Arg778Leu, was found in 32 (64%) patients, in at least one allele. The frequency of the other most prevalent mutation Pro992Leu was 27%. In the copper-binding domain, we found two novel mutations: one non-conservative, which replaced a highly conserved acidic amino acid with a small non-polar amino acid (Asp96Gly); and the other conservative (Asp196Glu), but lying at a highly conserved position in Menkes disease and other copper-transporting ATPases. The two novel mutations were both missense mutations, and the nucleotide changes were not found in the 100 normal individuals. The novel features of these mutations were identified according to the database that is maintained by the University of Alberta (http://www.medicalgenetics.med.ualberta.ca/wilson/index.php).

DNA polymorphism

Seven DNA sequence polymorphisms were identified (Table 2). DNA sequence polymorphisms were those nucleotide changes that either did not modify the amino acid sequence of the polypeptide, resulted in conservative changes in the amino acid residues, or that were found in normal chromosomes or chromosomes with known disease-causing mutant alleles. In order to clarify these nucleotide and amino acid sequence changes were mutations or polymorphisms, we analyzed 100 normal individuals with direct sequencing of their PCR products. The nucleotide changes present in those 100 normal individuals were considered to be polymorphisms, and those which were not present, were considered to be mutations.

Genotype-phenotype correlations

A total of 50 patients with WD were analyzed. The mean age of patients at disease manifestation was 8.9 ± 1.9 years (median, 8 years; range, 4-13 years). Thirty patients (60%) had a primary hepatic manifestation, 13 showed a primary neurological manifestation, and five had combined hepatic and neurological manifestations. Two patients (4%) identified by family screening had no symptoms; they were classified as asymptomatic, and were not included in analysis of genotype-phenotype correlations.

We identified 18 homozygotes and 14 heterozygotes for Arg778Leu. In the 32 patients carrying Arg778Leu, 25 (78.1%) had hepatic manifestations (age of onset, 8.1 ± 1.7 years). There were a total of 18 patients (36%) without the Arg778Leu mutation, 10 (55.6%) with hepatic manifestations (age of onset, 9.2 ± 2.3 years). The Arg778Leu homozygous patients were not significantly younger at the time of symptom onset (8.5 ± 2.1 years), compared with compound heterozygotes (7.9 ± 1.8

Table 1 Mutations identified in WD chromosomes

Mutation	Nucleotide change	Exon	Predicted effect	Frequency of WD alleles (%)	Frequency of WD alleles in Caucasian patients (%)
Asp96Gly	287A>G	2	Disrupts Cu 1	8	novel
Asp196Glu	588C>A	2	Disrupts Cu 2	2	novel
Arg778Leu[1]	2333G>T	8	Disrupts TM4	50	2
Pro992Leu[2]	2975C>T	13	Disrupts Ch/TM6	27	2
Val1216Met[3]	3646G>A	17	Disrupts ATP binding	3	< 1

[1]Mutation previously described[5]. [2]Mutation previously described[1]. [3]Mutation previously described[1].

Table 2 Polymorphism identified at the ATP7B locus

Polymorphism	Nucleotide change	Exon	Frequency (%)	
			Healthy individuals (n = 100)	Patients (n = 50)
-75 A > C[1]		5′UTR	38	10
-123 del CGCCG[1]		5′UTR	10	25
S406A[1]	1216 T>G	2	35	30
V456L[1]	1366 G>C	3	36	22
L770L[2]	2310 C>G	8	2	50
K832R[2]	2495 G>A	10	27	30
S1166S[3]	3498 C>T	16	19	20

[1]Polymorphism previously described[12]. [2]Polymorphism previously described[12]. [3]Polymorphism previously described[13].

years). However, the number of Eastern Chinese patients with hepatic manifestations among those carrying the Arg778Leu mutation was significantly greater than that among those without the mutation ($\chi^2 = 5.26$, $P < 0.05$).

DISCUSSION

Mutation analysis is important in the early diagnosis of patients with a family history of WD, as well as in prenatal diagnosis. Here, we report a group of 50 subjects affected with WD, analyzed by direct sequencing of the entire coding sequence of ATP7B gene. We found mutations in 50 patients. The detection rate of mutation was 92%. Seventy-seven percent of the mutations detected were lying in exons 8 and 13. According to this study, we recommend screening of exons 8 and 13 by sequence analysis. Mutations in exons 14 and 18, which have been found to have a high frequency among Caucasian patients[24-27], were not detected in our study. As reported previously[12], Arg778Leu was the most common WD chromosomal mutation detected in the present study (50%). However, Arg778Gln was not detected in our study, which is similar to the results of previous research in Shanghai[12]. The unusual high frequency of codon 778 mutation may have been due to sampling disequilibrium or to the presence of a founder effect among the Eastern Chinese population. Pro992Leu was the second most frequent allele, with a frequency of 27%. The above-mentioned two mutations accounted for ~80% of all mutations detected in our study. In eight patients, only one allelic change was detected, which suggests that the other change might be located in the introns or regulatory regions. Incomplete assessment of intronic and regulatory sequences may have accounted for the second unidentified mutation.

We could not find any significant difference between Arg778Leu homozygosity and heterozygosity with regard to the mean age of onset of symptoms, although Wu *et al*[7] have reported that the average age of onset in 18 Chinese homozygotes was significantly lower than that in 11 Chinese compound heterozygotes for Arg778Leu. However, the number of patients with hepatic manifestations among those carrying Arg778Leu mutation was significantly greater than that among patients without the mutation. Caca *et al*[28] have reported that symptoms in most His1069Gln homozygotes started between 16 and 25 years of age. In our study, symptoms in most Arg778Leu homozygotes (15/18) started before 10 years of age, which suggests that the age of onset in Arg778Leu homozygotes in Eastern Chinese patients is earlier than that in His1069Gln homozygotes in East German patients. His1069Gln is the most common WD mutation found in the European population[29-31], but it was not detected in our study in Eastern Chinese patients.

Molecular diagnosis of pre-symptomatic WD patients is important in the control of disease progression and treatment. Mutation detection in WD is challenging because of the presence of a large number of mutations in a 4.4-kb coding region in 21 exons spread over 80 kb of genomic DNA. The mutation detection rate among the WD chromosome was > 90% in our study. This gene sequencing analysis method was shown to have a high detection rate and accuracy. It may become the first priority in the screening of WD patients.

COMMENTS

Background

Direct mutation analysis has been performed in many WD patients, and more than 200 different ATP7B mutations have been detected.

Research frontiers

The mutations in the Chinese population with WD, and the possible correlation of specific mutations with clinical characteristics have not been investigated. In the present study, mutations of the ATP7B gene were sought by means of direct sequencing in 50 Eastern Chinese patients of Han ethnic origin with WD.

Innovations and breakthroughs

Two novel mutations were identified in our normal individuals. The novel features of the mutations were identified according to a database that is maintained by the University of Alberta.

Peer review

It is an interesting manuscript. The first concern is on the study population. The

subjects were from mainland Eastern China, which covers a large area. More specific information on subject source, which clinic they attended, and conditions under which they were recruited would be helpful.

REFERENCES

1 **Tsai CH,** Tsai FJ, Wu JY, Chang JG, Lee CC, Lin SP, Yang CF, Jong YJ, Lo MC. Mutation analysis of Wilson disease in Taiwan and description of six new mutations. *Hum Mutat* 1998; **12**: 370-376

2 **Gupta A**, Neogi R, Mukherjea M, Mukhopadhyay A, Roychoudhury S, Senapati A, Gangopadhyay PK, Ray K. DNA linkage based diagnosis of Wilson disease in asymptomatic siblings. *Indian J Med Res* 2003; **118**: 208-214

3 **Gupta A,** Aikath D, Neogi R, Datta S, Basu K, Maity B, Trivedi R, Ray J, Das SK, Gangopadhyay PK, Ray K. Molecular pathogenesis of Wilson disease: haplotype analysis, detection of prevalent mutations and genotype-phenotype correlation in Indian patients. *Hum Genet* 2005; **118**: 49-57

4 **Yarze JC,** Munoz SJ, Friedman LS. Diagnosing Wilson disease. *Ann Intern Med* 1992; **117**: 91

5 **Chuang LM**, Wu HP, Jang MH, Wang TR, Sue WC, Lin BJ, Cox DW, Tai TY. High frequency of two mutations in codon 778 in exon 8 of the ATP7B gene in Taiwanese families with Wilson disease. *J Med Genet* 1996; **33**: 521-523

6 **Waldenström E,** Lagerkvist A, Dahlman T, Westermark K, Landegren U. Efficient detection of mutations in Wilson disease by manifold sequencing. *Genomics* 1996; **37**: 303-309

7 **Wu ZY**, Lin MT, Murong SX, Wang N. Molecular diagnosis and prophylactic therapy for presymptomatic Chinese patients with Wilson disease. *Arch Neurol* 2003; **60**: 737-741

8 **Bull PC,** Thomas GR, Rommens JM, Forbes JR, Cox DW. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. *Nat Genet* 1993; **5**: 327-537

9 **Tanzi RE**, Petrukhin K, Chernov I, Pellequer JL, Wasco W, Ross B, Romano DM, Parano E, Pavone L, Brzustowicz LM. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nat Genet* 1993; **5**: 344-350

10 **Gitlin JD.** Wilson disease. *Gastroenterology* 2003; **125**: 1868-1877

11 **Shah AB,** Chernov I, Zhang HT, Ross BM, Das K, Lutsenko S, Parano E, Pavone L, Evgrafov O, Ivanova-Smolenskaya IA, Annerén G, Westermark K, Urrutia FH, Penchaszadeh GK, Sternlieb I, Scheinberg IH, Gilliam TC, Petrukhin K. Identification and analysis of mutations in the Wilson disease gene (ATP7B): population frequencies, genotype-phenotype correlation, and functional analyses. *Am J Hum Genet* 1997; **61**: 317-328

12 **Liu XQ,** Zhang YF, Liu TT, Gu XF, Hsiao KJ, Bao KR, Yu LH. Genotype and phenotype correlation in Chinese patients with Wilson's Disease. *Zhonghua Erke Zazhi* 2003; **41**: 35-38

13 **Al Jumah M**, Majumdar R, Al Rajeh S, Awada A, Al Zaben A, Al Traif I, Al Jumah AR, Rehana Z. A clinical and genetic study of 56 Saudi Wilson disease patients: identification of Saudi-specific mutations. *Eur J Neurol* 2004; **11**: 121-124

14 **Riordan SM**, Williams R. The Wilson's disease gene and phenotypic diversity. *J Hepatol* 2001; **34**: 165-171

15 **Ferenci P.** Regional distribution of mutations of the ATP7B gene in patients with Wilson disease: impact on genetic testing. *Hum Genet* 2006; **120**: 151-159

16 **Vrabelova S**, Letocha O, Borsky M, Kozak L. Mutation analysis of the ATP7B gene and genotype/phenotype correlation in 227 patients with Wilson disease. *Mol Genet Metab* 2005; **86**: 277-285

17 **Nanji MS,** Nguyen VT, Kawasoe JH, Inui K, Endo F, Nakajima T, Anezaki T, Cox DW. Haplotype and mutation analysis in Japanese patients with Wilson disease. *Am J Hum Genet* 1997; **60**: 1423-1429

18 **Kim EK,** Yoo OJ, Song KY, Yoo HW, Choi SY, Cho SW, Hahn SH. Identification of three novel mutations and a high frequency of the Arg778Leu mutation in Korean patients with Wilson disease. *Hum Mutat* 1998; **11**: 275-278

19 **Xu P,** Liang X, Jankovic J, Le W. Identification of a high frequency of mutation at exon 8 of the ATP7B gene in a Chinese population with Wilson disease by fluorescent PCR. *Arch Neurol* 2001; **58**: 1879-1882

20 **Liu XQ,** Zhang YF, Liu TT, Hsiao KJ, Zhang JM, Gu XF, Bao KR, Yu LH, Wang MX. Correlation of ATP7B genotype with phenotype in Chinese patients with Wilson disease. *World J Gastroenterol* 2004; **10**: 590-593

21 **Yoo HW.** Identification of novel mutations and the three most common mutations in the human ATP7B gene of Korean patients with Wilson disease. *Genet Med* 2002; **4**: 43S-48S

22 **Kusuda Y**, Hamaguchi K, Mori T, Shin R, Seike M, Sakata T. Novel mutations of the ATP7B gene in Japanese patients with Wilson disease. *J Hum Genet* 2000; **45**: 86-91

23 **Ferenci P,** Caca K, Loudianos G, Mieli-Vergani G, Tanner S, Sternlieb I, Schilsky M, Cox D, Berr F. Diagnosis and phenotypic classification of Wilson disease. *Liver Int* 2003; **23**: 139-142

24 **Olivarez L**, Caggana M, Pass KA, Ferguson P, Brewer GJ. Estimate of the frequency of Wilson's disease in the US Caucasian population: a mutation analysis approach. *Ann Hum Genet* 2001; **65**: 459-463

25 **Chappuis P,** Callebert J, Quignon V, Woimant F, Laplanche JL. Late neurological presentations of Wilson disease patients in French population and identification of 8 novel mutations in the ATP7B gene. *J Trace Elem Med Biol* 2007; **21**: 37-42

26 **Gromadzka G**, Schmidt HH, Genschel J, Bochow B, Rodo M, Tarnacka B, Litwin T, Chabik G, Członkowska A. p.H1069Q mutation in ATP7B and biochemical parameters of copper metabolism and clinical manifestation of Wilson's disease. *Mov Disord* 2006; **21**: 245-248

27 **Panagiotakaki E,** Tzetis M, Manolaki N, Loudianos G, Papatheodorou A, Manesis E, Nousia-Arvanitakis S, Syriopoulou V, Kanavakis E. Genotype-phenotype correlations for a wide spectrum of mutations in the Wilson disease gene (ATP7B). *Am J Med Genet A* 2004; **131**: 168-173

28 **Caca K,** Ferenci P, Kühn HJ, Polli C, Willgerodt H, Kunath B, Hermann W, Mössner J, Berr F. High prevalence of the H1069Q mutation in East German patients with Wilson disease: rapid detection of mutations by limited sequencing and phenotype-genotype analysis. *J Hepatol* 2001; **35**: 575-581

29 **Thomas GR**, Forbes JR, Roberts EA, Walshe JM, Cox DW. The Wilson disease gene: spectrum of mutations and their consequences. *Nat Genet* 1995; **9**: 210-217

30 **Folhoffer A**, Ferenci P, Csak T, Horvath A, Hegedus D, Firneisz G, Osztovits J, Kosa JP, Willheim-Polli C, Szonyi L, Abonyi M, Lakatos PL, Szalay F. Novel mutations of the ATP7B gene among 109 Hungarian patients with Wilson's disease. *Eur J Gastroenterol Hepatol* 2007; **19**: 105-111

31 **Brage A,** Tomé S, García A, Carracedo A, Salas A. Clinical and molecular characterization of Wilson disease in Spanish patients. *Hepatol Res* 2007; **37**: 18-26

S- Editor Liu Y **L- Editor** Kerr C **E- Editor** Li HY

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5151-5153
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Sirolimus-related pulmonary toxicity mimicking 'asthma like' symptoms

GL Gupte, S Mahadevan, JR Clarke, H Alton, SV Beath

GL Gupte, S Mahadevan, SV Beath, Liver Unit, Birmingham Children's Hospital, Steelhouse Lane, B4 6NH, United Kingdom
JR Clarke, Department of Respiratory Medicine, Birmingham Children's Hospital, Steelhouse Lane, B4 6NH, United Kingdom
H Alton, Department of Radiology, Birmingham Children's Hospital, Steelhouse Lane, B4 6NH, United Kingdom
Correspondence to: Dr. Girish Gupte, Consultant Paediatric Hepatologist, Birmingham Children's Hospital, Steelhouse Lane, Birmingham B4 6NH,
United Kingdom. girish.gupte@bch.nhs.uk
Telephone: + 44-121-3338267 Fax: + 44-121-3338251
Received: June 12, 2007 Revised: August 1, 2007

Abstract

Sirolimus is an immunosuppressant with expanding use in pediatric organ transplantation, dermatology and rheumatology. We report two cases of children who developed asthma like symptoms and were diagnosed with interstitial lung disease, which responded to discontinuation of sirolimus. Pediatricians should be aware about the pulmonary side effects of sirolimus.

Key words: Sirolimus; Pulmonary toxicity; Interstitial lung disease; Asthma; Small bowel transplantation; Intestinal transplantation; Organ transplantation

Gupte GL, Mahadevan S, Clarke JR, Alton H, Beath SV. Sirolimus related pulmonary toxicity mimicking 'asthma like' symptoms. *World J Gastroenterol* 2007; 13(38): 5151-5153

http://www.wjgnet.com/1007-9327/13/5151.asp

INTRODUCTION

Sirolimus (rapamycin) is a macrolide immunosuppressant with increasing use in various pediatric subspecialities[1,2]. Its dose-related side effects leading to thrombocytopenia and hypercholesterolemia are widely known. Pulmonary toxicity associated with sirolimus therapy has only been recently recognized as a potentially serious complication[3]. Among the twenty-eight children (18 with liver transplant and 10 with small bowel transplant) treated with sirolimus in our center, two developed pulmonary complications which are described in this case report.

CASE REPORT

Case 1

A 2-year-old girl with microvillous inclusion disease underwent combined small bowel and liver transplantation (CSBLTx). She received tacrolimus and prednisolone according to our immunosuppression protocol. She developed severe rejection at the age of 2 years due to poor compliance with medications. Sirolimus (0.1 mg/kg per day) was added to her immunosuppression regime aiming at levels of 8-10 ng/mL for both tacrolimus and sirolimus.

Four months after starting sirolimus treatment, she developed a perforation near the anastomotic site of the intestinal allograft and sirolimus was stopped. She remained generally well in the next year, but in view of deteriorating renal function, and persistent hypomagnesaemia, the tacrolimus dose was halved (target level 3-5 ng/mL) and sirolimus was added (target level 8-10 ng/mL). Two months after commencing sirolimus she acquired adenovirus infection which required mechanical ventilation and reduction of immunosuppression (target level of tacrolimus and sirolimus 3-5 ng/mL). After the reduction of immunosuppression, she showed an uneventful recovery.

During the next 5 mo (now 4 years and 9 mo after transplantation), in which sirolimus was re-introduced, she developed a dry cough and increased respiratory rate with mild subcostal recession. Her clinical examination was otherwise unremarkable. Initially she had no response to bronchodilators for possible asthma. Pulmonary function tests were normal. Her chest X-ray showed some loss of volume in the left lower lobe and coarse interstitial shadowing throughout both lung fields. Immunofluorescence and viral culture of nasopharyngeal aspirates and broncho-alveolar lavage (BAL) including adenovirus were all negative. The BAL fluid grew haemophilus influenzae, which was treated with amoxicillin. Blood cultures for fungi and bacteria were negative, as was PCR for CMV and EBV. A high resolution CT scan of the chest demonstrated fine nodular changes in the interstitial air spaces. A lung biopsy was considered but deferred. Sirolimus-associated pulmonary toxicity was suspected and sirolimus was discontinued. Her respiratory symptoms of cough and dyspnea improved within 3 mo and her chest X-ray changes resolved within 6 mo.

Case 2

A 9-mo-old boy with Hirschprung disease with

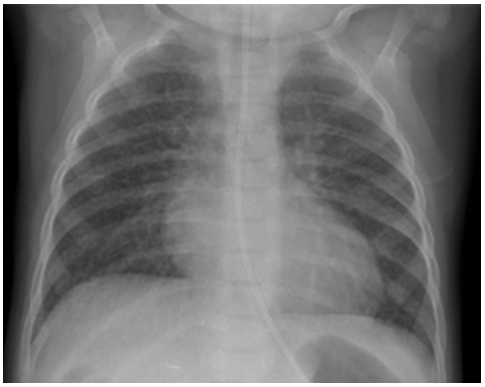


Figure 1 Chest X-ray showing bilateral interstitial shadowing and nodularity.

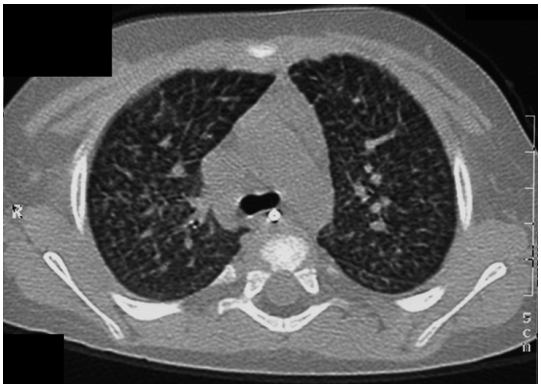


Figure 2 CT scan of the chest showing thickening of the interlobular septae with fine nodular changes.

total aganglionosis extending proximally to the pylorus underwent CSBLTx. He was commenced on tacrolimus and prednisolone according to our standard immunosuppression protocol. An episode of moderate acute rejection 10 d after transplant was treated with a single dose of steroids and two doses of basiliximab four days apart. Sirolimus (0.14 mg/kg) was commenced 6 wk after transplant. The aim was to maintain tacrolimus and sirolimus levels at 8-10 ng/mL and 6-8 ng/mL, respectively. Five months after commencing sirolimus, he showed features of bone marrow suppression with neutropenia and anemia. A bone marrow aspirate showed myeloid maturation arrest and responded well to weekly injection of granulocyte colony stimulating factor for 3 mo and supportive treatment.

In view of bone marrow suppression, the dose of sirolimus was reduced to maintain a level 4-6 ng/mL. Seven months after introduction of sirolimus, he presented with a persistent dry cough and breathlessness on exertion. Respiratory examination was normal. Chest X-ray showed bilateral interstitial shadowing and nodularity (Figure 1), but overnight pulse oximetry observation was normal. A comprehensive evaluation for post-transplant lymphoproliferative disease (PTLD), including biopsies from upper GI endoscopy, liver biopsy, and ileoscopy was negative. EBV PCR titer was low with a level of 1000 genome copies/mL. Investigations for respiratory infection, including BAL, revealed no infection and special staining for pneumocystis carinii pneumonia, acid-fast bacilli staining and immunochemistry for cytomegalovirus and Epstein-Barr virus were negative. A high resolution CT scan revealed thickening of the interlobular septae with fine nodular changes (Figure 2). A lung biopsy was considered and deferred. Following discontinuation of sirolimus, his symptoms resolved within 4 wk, but radiological changes were not resolved after 18 mo of follow-up.

DISCUSSION

The resolution of clinical symptoms and improvement in radiological changes after withdrawal of sirolimus in our patients, with the absence of other infectious factors, strongly implicates that sirolimus is the causative factor for the pulmonary changes.

Sirolimus-associated pulmonary toxicity is mostly described in the adult literature and actually represents a spectrum of clinico-pathologic syndromes characterized clinically by dyspnea, cough, fever, fatigue or haemoptysis, and histologically by the presence of organizing pneumonia, interstitial pneumonitis, focal fibrosis or by the presence of alveolar haemorrhage[4]. In the largest report on sirolimus-associated pulmonary toxicity in adult kidney transplant recipients, features of pneumonitis were seen within 6-12 mo after commencing sirolimus therapy[3]. Our patients showed a similar time course .

The children in this report exhibited 'asthma like' symptoms with recurrent episodes of dry cough and breathlessness on exertion. In children on immunosuppressive drugs, the diagnostic challenge is to rule out opportunistic infections. Lung biopsy looking for any histological changes was not performed due to the invasive nature of the procedure and resolution of the symptoms and radiological changes on cessation of sirolimus. A review of drug history did not identify any other medicines in the complex cases, which could give rise to the 'asthma' like symptoms.

The management consists of excluding other etiologies, especially opportunistic infections, and discontinuation or dose reduction of sirolimus[3]. As in the previous reports, respiratory symptoms resolved within 2-4 wk after cessation of sirolimus therapy with improvement of CXR changes in 6-18 mo, which is consistent with other case reports[3,5]. We opted to stop the treatment as the long-term outcome of interstitial lung changes in children is not known. It is possible that the degree of reversal of pulmonary symptoms depends on the extent and the chronicity of parenchymal and interstitial changes. The children reported were on low dose steroids as a part of their immunosupression regime and developed interstitial lung disease despite being on steroids.

The exact pathogenic mechanism of sirolimus-induced pulmonary toxicity is not known. Possible mechanisms include idiosyncratic cell-mediated autoimmune response due to the exposure of cryptic antigens and T cell-mediated delayed type hypersensitivity reaction[3,6]. Of the 28 children (10 with small bowel transplant, 18 with liver transplant) treated with sirolimus in the liver unit at BCH, two children with small bowel transplant developed the

changes, while none of the children with liver transplants developed this complication. It is entirely possible that the complication may be related to the higher intensity of immunosuppression used in the children with intestinal transplantation. However, a simple dose-dependant toxicity reaction seems less likely since other dose-dependent side effects such as thrombocytopenia or hypercholesterolemia were absent. Morath C *et al*[7] have documented that an increase in sirolimus levels 3 wk prior to the onset of symptoms and an older age are the risk factors for developing interstitial lung disease. Similar observations could not be made from our two cases.

In conclusion, with the expanding use of sirolimus in children, the appearance of persistent respiratory symptoms, especially cough and dyspnea, should alert the pediatrician to the possibility of sirolimus-associated pulmonary toxicity and the drug may have to be discontinued.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the help from Dr. M Sood (Paediatric Gastroenterologist, Booth Hall Children's Hospital, Manchester) and Dr. S Mitton (Paediatric Gastroenterologist, St George's Hospital, London) in the shared care of these complex patients.

REFERENCES

1 **Marsland AM**, Griffiths CE. The macrolide immunosuppressants in dermatology: mechanisms of action. *Eur J Dermatol* 2002; **12**: 618-622
2 **Drosos AA**. Newer immunosuppressive drugs: their potential role in rheumatoid arthritis therapy. *Drugs* 2002; **62**: 891-907
3 **Pham PT**, Pham PC, Danovitch GM, Ross DJ, Gritsch HA, Kendrick EA, Singer J, Shah T, Wilkinson AH. Sirolimus-associated pulmonary toxicity. *Transplantation* 2004; **77**: 1215-1220
4 **Singer SJ**, Tiernan R, Sullivan EJ. Interstitial pneumonitis associated with sirolimus therapy in renal-transplant recipients. *N Engl J Med* 2000; **343**: 1815-1816
5 **Lennon A**, Finan K, FitzGerald MX, McCormick PA. Interstitial pneumonitis associated with sirolimus (rapamycin) therapy after liver transplantation. *Transplantation* 2001; **72**: 1166-1167
6 **Morelon E**, Stern M, Israël-Biet D, Correas JM, Danel C, Mamzer-Bruneel MF, Peraldi MN, Kreis H. Characteristics of sirolimus-associated interstitial pneumonitis in renal transplant patients. *Transplantation* 2001; **72**: 787-790
7 **Morath C**, Schwenger V, Ksoll-Rudek D, Sommerer C, Beimler J, Schmidt J, Zeier M. Four cases of sirolimus-associated interstitial pneumonitis: identification of risk factors. *Transplant Proc* 2007; **39**: 99-102

S- Editor Liu Y **L- Editor** Wang XL **E- Editor** Lu W

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5154-5157
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Upper gastrointestinal bleeding from duodenal vascular ectasia in a patient with cirrhosis

Beom Jae Lee, Jong-Jae Park, Yeon Seok Seo, Ji Hoon Kim, Aeree Kim, Jong Eun Yeon, Jae Seon Kim, Kwan Soo Byun, Young-Tae Bak

Beom Jae Lee, Jong-Jae Park, Yeon Seok Seo, Ji Hoon Kim, Aeree Kim, Jong Eun Yeon, Jae Seon Kim, Kwan Soo Byun, Young-Tae Bak, Division of Gastroenterology, Department of Internal Medicine, Korea University College of Medicine, Seoul, Korea
Aeree Kim, Department of Pathology, College of Medicine, Korea University, Seoul 152-703, Korea
Correspondence to: Jong-Jae Park, MD, PhD, Division of Gastroenterology, Department of Internal Medicine, Korea University Guro Hospital, Gurodong-gil 97, Guro-gu, Seoul 152-703, Korea. gi7pjj@yahoo.co.kr
Telephone: +82-2-8186637 Fax: +82-2-8371966
Received: May 30, 2007 Revised: June 18, 2007

Abstract

We report a cirrhotic patient with duodenal vascular ectasia and spontaneous bleeding. The bleeding was successfully controlled with argon plasma coagulation. Duodenal vascular ectasia may be a cause of upper gastrointestinal bleeding in patients with cirrhosis, and argon plasma coagulation may be effective and safe to achieve hemostasis of this lesion.

Key words: Vascular ectasia; Duodenum; Cirrhosis; Gastrointestinal hemorrhage; Hemostasis

Lee BJ, Park JJ, Seo YS, Kim JH, Kim A, Yeon JE, Kim JS, Byun KS, Bak YT. Upper gasgtrointestinal bleeding from duodenal vascular ectasia in a patient with cirrhosis. *World J Gastroenterol* 2007; 13(38): 5154-5157

http://www.wjgnet.com/1007-9327/13/5154.asp

INTRODUCTION

Vascular ectasia of the duodenum is rare[1] and an uncommon source of upper gastroduodenal bleeding. It is associated with chronic illnesses such as aortic valve disease and end-stage renal disease when patients are under long-term hemodialysis[2-4]. Gallagher *et al*[5] reported a patient with small bowel capillary dilatation and cirrhosis, and suggested that small bowel capillary dilatation appears to be unique in patients with portal hypertension, and may play a role in causing gastrointestinal bleeding. However, to our knowledge, only a few cases of duodenal vascular ectasia have been reported in patients with cirrhosis[5-7]. We report a case of upper gastrointestinal bleeding in a patient with cirrhosis and duodenal vascular ectasia, which was successfully controlled by argon plasma coagulation (APC).

CASE REPORT

A 62-year-old man was admitted because of melena, dizziness, and anemia with a follow-up history for chronic hepatitis B infection, liver cirrhosis, and hepatocellular carcinoma. About a 2 cm-sized tumor mass was located in subcapsular portion of segment 7. After two sessions of transarterial chemoembolization for the tumor mass, no visible tumor was found. He visited the emergency room two years ago because of hematemesis, and an endoscopic examination revealed esophageal varices with bleeding, which were treated with endoscopic band ligation.

Physical examination revealed blood pressure of 100/80 mmHg, pulse rate of 72 beats per minute, respiratory rate of 14 breaths per minute, and body temperature of 36.8℃. He was alert and oriented to person, place, and time. His sclera was nonicteric and conjunctiva was pale. Chest and heart examinations were normal. Abdominal examination revealed a distended abdomen with prominent shifting dullness. Pitting edema was noted in both lower extremities.

The results of initial laboratory tests were as follows: white blood count (WBC) = 3700/mm^3, hemoglobin = 7.3 g/dL, platelets = 34000/mm^3, prothrombin time = 23.8 s, international normalized ratio = 2.05, C-reactive protein = 82.6 mg/dL, total bilirubin= 2.07 mg/dL, direct bilirubin = 0.96 mg/dL, protein = 5.23 g/dL, albumin = 2.83 g/dL, alanine aminotransferase = 67 IU/L, aspartate aminotransferase = 62 IU/L, gamma-glucuronyl transferase = 38 IU/L, alkaline phosphatase = 87 IU/L, glucose = 142 mg/dL, blood urea nitrogen (BUN)/creatine(Cr) = 19.57/0.75 mg/dL, and Na/K/Cl = 120/4.1/87 mEq/mL. Paracentesis revealed clear yellow-colored ascitic fluid, which on analysis showed 0/mm^3 WBC, 2000/mm^3 red blood cells, 242 mg/dL glucose, and 252 mg/dL protein. The serum α-fetoprotein concentration was 6.2 ng/mL. He was positive for hepatitis B surface antigen and negative for hepatitis B envelope antigen.

Bleeding from the esophageal varices was suspected and urgent endoscopic examination was performed. However, the urgent endoscopy showed small esophageal varices

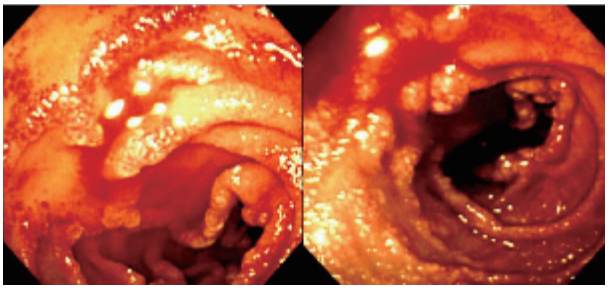


Figure 1 Endoscopy showing a vascular ectasia with active bleeding on the second portion of duodenum.

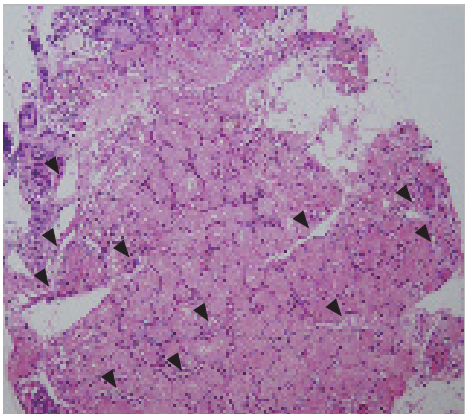


Figure 2 Biopsy from duodenal mucosa showing grossly dilated blood vessels (arrowheads) in the Brunner's glands (HE, × 100).

with no evidence of bleeding, and unexpectedly, vascular ectasia with oozing was found on the duodenal bulb and second portion (Figure 1). Endoscopic biopsy was performed on the vascular ecstatic mucosa and histological examination revealed grossly dilated blood vessels in the Brunner's glands at the mucosal layer (Figure 2). To achieve hemostasis, APC (Arco-2000, Soring, Germany) was performed (argon gas flow = 1.5 L/min, power = 50 W), which controlled the bleeding without any complications such as bowel perforation (Figure 3). Follow-up endoscopic examination after one week showed minimal blood oozing on the telangiectatic duodenal mucosa, and a second session of APC was performed (Figure 4A). After endoscopic treatment, his hemoglobin concentration did not decrease and his symptoms improved, and the patient was discharged.

Two months after discharge, endoscopic examination revealed a remnant vascular ectatic duodenal mucosa but no spontaneous bleeding (Figure 4B).

DISCUSSION

Upper gastrointestinal bleeding is one of the most frequent causes of morbidity and mortality during the clinical course of liver cirrhosis[8-11] and has an overall mortality rate of 24% at six weeks and 40% at one year[12]. A recent study showed that gastroesophageal varices (59.1%) comprise the most frequent bleeding lesion in patients with cirrhosis[13]. Others have reported a similar frequency

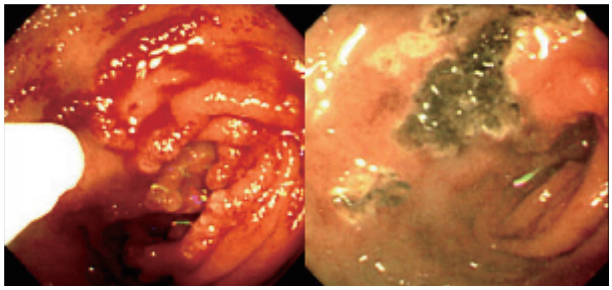


Figure 3 Induction of hemostasis by APC (Arco-2000, Soring, Germany) (left) with blood oozing controlled without complications (right).

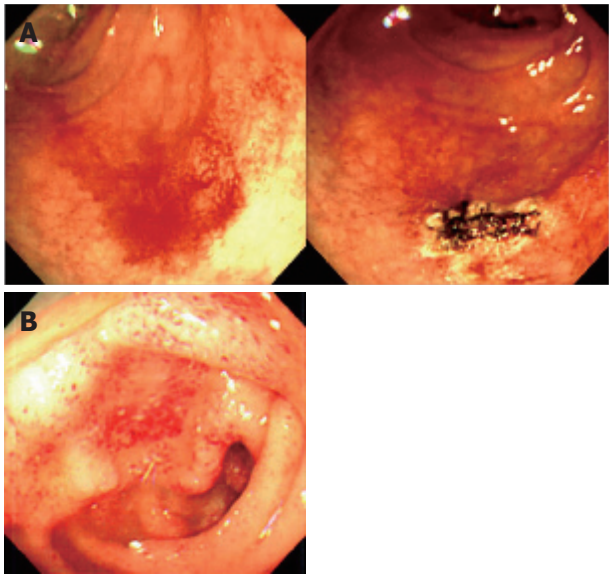


Figure 4 A one-week follow-up endoscopy showing the successfully controlled blood oozing from vascular ectasia on the second portion of the duodenum with APC (**A**) and a two-month follow-up endoscopy showing the ectatic vascular duodenal mucosa with no further spontaneous bleeding (**B**).

of esophageal varices bleeding, ranging 49%-72%[12,14,15]. Peptic ulcer bleeding is the second most frequent type of bleeding lesion (15.7%)[13].

Upper gastrointestinal vascular ectasia is recognized as an increasingly important source of gastrointestinal bleeding, and can present as that of either overt or of an obscure cause[3,16-19]. Gastric antral vascular ectasia (GAVE) is a distinct vascular abnormality involving mainly the gastric antrum. Endoscopic examination shows linear, friable red streaks radiating from the pylorus. Angiodysplastic lesions are typically discrete, flat, or slightly raised bright-red lesions 2-10 mm in size, often with fern-like margins and a surrounding pale rim[20]. The clinical presentation is either chronic, low-grade bleeding, which often leads to iron-deficiency anemia, or acute bleeding with hematemesis or melena. Patients may have recurrent episodes of bleeding which require multiple transfusions, and become transfusion dependent[20]. A recent study showed that 29% of patients with vascular ectasia also have chronic liver diseases[21].

By definition, GAVE occurs only in the antrum. However, ectopic ectasia has been reported in the cardiac region[22], gastric corpus[23], duodenum[22,24], and possibly in

the colon[22]. Astaldi and Strosseli[25] suggested that small bowel biopsies from patients with hepatic cirrhosis might show vascular stasis arising from portal stasis. Bank *et al*[26] have noted a 'striking increase in vascularity of the villi' in a small bowel biopsy of a patient with cirrhosis and esophageal varices. Gallagher *et al*[5] suggested that capillary dilatation is probably caused by increased pressure in the portal system.

To our knowledge, only a few cases of vascular ectasia of the duodenum have been reported in patients with cirrhosis[5-7]. In one report, histology of the endoscopic duodenal biopsy revealed grossly dilated capillaries in the villi of the duodenum and jejunum, the patient received a portacaval shunt, but died postoperatively[5]. Arendse *et al*[6] reported a fatal case of vascular ectasia of the duodenum in a patient with cirrhosis, which was revealed by autopsy. Cales *et al*[7] reported an ectasia in a patient with cirrhosis, which was revealed by autopsy. The patient was treated with endoscopic sclerotherapy and a portosystemic shunt to control the bleeding, although neither was successful. Our study seems to be the first to report successful control of bleeding from vascular ectasia of the duodenum by APC in a patient with cirrhosis.

We attempted to treat bleeding from duodenal vascular ectasia by APC and found a certain improvement on endoscopic examination of the duodenum. However, we did not eradicate the duodenal vascular ectasia. Pavey *et al*[20] reported that patients with GAVE require more sessions to fully control the bleeding and more transfusion than patients with angiodysplasia. Our patient also seemed to need more sessions of APC to completely eradicate ectasia, although this was not possible because he did not visit our hospital after the last endoscopic examination.

Before the widespread availability of therapeutic endoscopy, vascular ectasia was treated with surgical resection[27,28]. More recently, upper gastrointestinal vascular ectasiae have been treated with various endoscopic techniques including heater probe coagulation[29], bipolar electrocautery[30] and Nd: YAG laser[31-33], and APC[20]. APC is a no-touch electrocoagulation technique in which a high-frequency monopolar alternating current is delivered to the tissue through ionized argon gas[34,35]. The advantages of this method include the technical ease in treating large areas and the ability to achieve superficial coagulation with a controllable depth of injury. The treated areas heal faster and the endpoint of therapy can be reached sooner[34,35]. In our patient, to reduce the risk of bowel perforation, we applied irradiation for 4-6 s with a current of 50 W, and were able to successfully control the bleeding without any serious complications.

In conclusion, duodenal vascular ectasia may be a cause of upper gastrointestinal bleeding in patients with cirrhosis. APC can be used as an effective and safe therapeutic tool to achieve hemostasis of this lesion.

REFERENCES

1 **Tai DI**, Chou FF, Lee TY, Lin CC. Vascular ectasia of the duodenum detected by duodenoscopy. *Am J Gastroenterol* 1987; **82**: 1071-1073

2 **Weaver GA**, Alpern HD, Davis JS, Ramsey WH, Reichelderfer M. Gastrointestinal angiodysplasia associated with aortic valve disease: part of a spectrum of angiodysplasia of the gut. *Gastroenterology* 1979; **77**: 1-11

3 **Clouse RE**, Costigan DJ, Mills BA, Zuckerman GR. Angiodysplasia as a cause of upper gastrointestinal bleeding. *Arch Intern Med* 1985; **145**: 458-461

4 **Cappell MS**, Lebwohl O. Cessation of recurrent bleeding from gastrointestinal angiodysplasias after aortic valve replacement. *Ann Intern Med* 1986; **105**: 54-57

5 **Gallagher C**, Bonar F, Dempsey J, Crowe J. Small bowel capillary dilatation in portal hypertension. *Postgrad Med J* 1985; **61**: 541-543

6 **Arendse MP**, Jaskiewicz K, Funnell I. Massive gastro-intestinal haemorrhage due to vascular ectasia of the duodenum. A case report. *S Afr J Surg* 1992; **30**: 111-113

7 **Calès P**, Voigt JJ, Payen JL, Bloom E, Berg P, Vinel JP, Pradère B, Broussy P, Pascal JP. Diffuse vascular ectasia of the antrum, duodenum, and jejunum in a patient with nodular regenerative hyperplasia. Lack of response to portosystemic shunt or gastrectomy. *Gut* 1993; **34**: 558-561

8 **Franco D**, Durandy Y, Deporte A, Bismuth H. Upper gastrointestinal haemorrhage in hepatic cirrhosis: causes and relation to hepatic failure and stress. *Lancet* 1977; **1**: 218-220

9 **Graham DY**, Smith JL. The course of patients after variceal hemorrhage. *Gastroenterology* 1981; **80**: 800-809

10 **Christensen E**, Krintel JJ, Hansen SM, Johansen JK, Juhl E. Prognosis after the first episode of gastrointestinal bleeding or coma in cirrhosis. Survival and prognostic factors. *Scand J Gastroenterol* 1989; **24**: 999-1006

11 **Burroughs AK**, Mezzanotte G, Phillips A, McCormick PA, McIntyre N. Cirrhotics with variceal hemorrhage: the importance of the time interval between admission and the start of analysis for survival and rebleeding rates. *Hepatology* 1989; **9**: 801-807

12 **del Olmo JA**, Peña A, Serra MA, Wassel AH, Benages A, Rodrigo JM. Predictors of morbidity and mortality after the first episode of upper gastrointestinal bleeding in liver cirrhosis. *J Hepatol* 2000; **32**: 19-24

13 **Lecleire S**, Di Fiore F, Merle V, Hervé S, Duhamel C, Rudelli A, Nousbaum JB, Amouretti M, Dupas JL, Gouerou H, Czernichow P, Lerebours E. Acute upper gastrointestinal bleeding in patients with liver cirrhosis and in noncirrhotic patients: epidemiology and predictive factors of mortality in a prospective multicenter population-based study. *J Clin Gastroenterol* 2005; **39**: 321-327

14 **Gatta A**, Merkel C, Amodio P, Bellon S, Bellumat A, Bolognesi M, Borsato L, Buttò M, Casson FF, Cavallarin G. Development and validation of a prognostic index predicting death after upper gastrointestinal bleeding in patients with liver cirrhosis: a multicenter study. *Am J Gastroenterol* 1994; **89**: 1528-1536

15 **D'Amico G**, De Franchis R. Upper digestive bleeding in cirrhosis. Post-therapeutic outcome and prognostic indicators. *Hepatology* 2003; **38**: 599-612

16 **Jensen DM**. Current diagnosis and treatment of severe obscure GI hemorrhage. *Gastrointest Endosc* 2003; **58**: 256-266

17 **Gretz JE**, Achem SR. The watermelon stomach: clinical presentation, diagnosis, and treatment. *Am J Gastroenterol* 1998; **93**: 890-895

18 **Abedi M**, Haber GB. Watermelon stomach. *Gastroenterologist* 1997; **5**: 179-184

19 **Jabbari M**, Cherry R, Lough JO, Daly DS, Kinnear DG, Goresky CA. Gastric antral vascular ectasia: the watermelon stomach. *Gastroenterology* 1984; **87**: 1165-1170

20 **Pavey DA**, Craig PI. Endoscopic therapy for upper-GI vascular ectasias. *Gastrointest Endosc* 2004; **59**: 233-238

21 **Lonardo A**, Greco M, Grisendi A. Bleeding gastrointestinal angiodysplasias: our experience and a review of the literature. *Ann Ital Med Int* 2004; **19**: 122-127

22 **Lux G**, Rosch W. Diffuse telangiectasia of the upper GI-tract. [Abstract]. *Endoscopy* 1979; **11**: 281

23 **Quintero E**, Pique JM, Bombi JA, Bordas JM, Sentis J, Elena M, Bosch J, Rodes J. Gastric mucosal vascular ectasias causing bleeding in cirrhosis. A distinct entity associated with hypergastrinemia and low serum levels of pepsinogen I.

Gastroenterology 1987; **93**: 1054-1061
24 **Wheeler MH**, Smith PM, Cotton PB, Evans DM, Lawrie BW. Abnormal blood vessels in the gastric antrum: a cause of upper-gastrointestinal bleeding. *Dig Dis Sci* 1979; **24**: 155-158
25 **Astaldi G**, Strosselli E. Peroral biopsy of the intestinal mucosa in hepatic cirrhosis. *Am J Dig Dis* 1960; **5**: 603-612
26 **Bank S**, Marks IN, Moshal MG, TIMME A. Peroral Intestinal Biopsy. Analysis Of Results In 134 Patients. *S Afr Med J* 1964; **38**: 451-458
27 **Moore JD**, Thompson NW, Appelman HD, Foley D. Arteriovenous malformations of the gastrointestinal tract. *Arch Surg* 1976; **111**: 381-389
28 **Cavett CM**, Selby JH, Hamilton JL, Williamson JW. Arteriovenous malformation in chronic gastrointestinal bleeding. *Ann Surg* 1977; **185**: 116-121
29 **Petrini JL Jr**, Johnston JH. Heat probe treatment for antral vascular ectasia. *Gastrointest Endosc* 1989; **35**: 324-328
30 **Binmoeller KF**, Katon RM. Bipolar electrocoagulation for watermelon stomach. *Gastrointest Endosc* 1990; **36**: 399-402
31 **Liberski SM**, McGarrity TJ, Hartle RJ, Varano V, Reynolds D. The watermelon stomach: long-term outcome in patients treated with Nd:YAG laser therapy. *Gastrointest Endosc* 1994; **40**: 584-587
32 **Tsai HH**, Smith J, Danesh BJ. Successful control of bleeding from gastric antral vascular ectasia (watermelon stomach) by laser photocoagulation. *Gut* 1991; **32**: 93-94
33 **Gostout CJ**, Ahlquist DA, Radford CM, Viggiano TR, Bowyer BA, Balm RK. Endoscopic laser therapy for watermelon stomach. *Gastroenterology* 1989; **96**: 1462-1465
34 **Johanns W**, Luis W, Janssen J, Kahl S, Greiner L. Argon plasma coagulation (APC) in gastroenterology: experimental and clinical experiences. *Eur J Gastroenterol Hepatol* 1997; **9**: 581-587
35 **Cipolletta L**, Bianco MA, Rotondano G, Piscopo R, Prisco A, Garofano ML. Prospective comparison of argon plasma coagulator and heater probe in the endoscopic treatment of major peptic ulcer bleeding. *Gastrointest Endosc* 1998; **48**: 191-195

S- Editor Zhu LH **L- Editor** Wang XL **E- Editor** Wang HF

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5158-5163
World Journal of Gastroenterology ISSN 1007-9327

CASE REPORT

Endoscopic ultrasound-guided fine-needle aspiration cytology diagnosis of solid pseudopapillary tumor of the pancreas: A case report and literature review

Charitini Salla, Paschalis Chatzipantelis, Panagiotis Konstantinou, Ioannis Karoumpalis, Akrivi Pantazopoulou, Victoria Dappola

Charitini Salla, Panagiotis Konstantinou, Akrivi Pantazopoulou, Victoria Dappola, Department of Cytology, Athens General Hospital, Athens 11635, Greece
Paschalis Chatzipantelis, Department of Pathology, Areteion University Hospital, Athens 11635, Greece
Ioannis Karoumpalis, Department of Gastroenterology, Athens General Hospital, Athens 11635, Greece
Correspondence to: Paschalis Chatzipantelis, Department of Pathology, Areteion University Hospital, 6-8 Stasinou Street, Athens 11635, Greece. pchatzipantelis@yahoo.com
Telephone: +30-210-7259424 Fax: +30-210-9480375
Received: June 4, 2007 Revised: July 28, 2007

Abstract

We describe the clinical, imaging and cytopathological features of solid pseudopapillary tumor of the pancreas (SPTP) diagnosed by endoscopic ultrasound-guided (EUS-guided) fine-needle aspiration (FNA). A 17-year-old woman was admitted to our hospital with complaints of an unexplained episodic abdominal pain for 2 mo and a short history of hypertension in the endocrinology clinic. Clinical laboratory examinations revealed polycystic ovary syndrome, splenomegaly and low serum amylase and carcinoembryonic antigen (CEA) levels. Computed tomography (CT) analysis revealed a mass of the pancreatic tail with solid and cystic consistency. EUS confirmed the mass, both in body and tail of the pancreas, with distinct borders, which caused dilation of the peripheral part of the pancreatic duct (major diameter 3.7 mm). The patient underwent EUS-FNA. EUS-FNA cytology specimens consisted of single cells and aggregates of uniform malignant cells, forming microadenoid structures, branching, papillary clusters with delicate fibrovascular cores and nuclear overlapping. Naked capillaries were also seen. The nuclei of malignant cells were round or oval, eccentric with fine granular chromatin, small nucleoli and nuclear grooves in some of them. The malignant cells were periodic acid Schiff (PAS)-Alcian blue (+) and immunocytochemically they were vimentin (+), CA 19.9 (+), synaptophysin (+), chromogranin (-), neuro-specific enolase (-), a1-antitrypsin and a1-antichymotrypsin focal positive. Cytologic findings were strongly suggestive of SPTP. Biopsy confirmed the above cytologic diagnosis. EUS-guided FNA diagnosis of SPTP is accurate. EUS findings, cytomorphologic features and immunostains of cell block help distinguish SPTP from pancreatic endocrine tumors, acinar cell carcinoma and papillary mucinous carcinoma.

Key words: Endosonography; Fine-needle aspiration; Solid pseudopapillary tumor; Pancreas; Cytology

Salla C, Chatzipantelis P, Konstantinou P, Karoumpalis I, Pantazopoulou A, Dappola V. Endoscopic ultrasound-guided fine-needle aspiration cytology diagnosis of solid pseudopapillary tumor of the pancreas: A case report and literature review. *World J Gastroenterol* 2007; 13(38): 5158-5163

http://www.wjgnet.com/1007-9327/13/5158.asp

INTRODUCTION

Solid pseudopapillary tumor of the pancreas (SPTP) is a rare neoplasm with a reported frequency of between 0.17% and 2.7% of all nonendocrine tumors of the pancreas[1], and 6.5% of all pancreatic tumors and tumor-like lesions resected in one large institute[2]. This tumor seems to preferentially occur in young females with a reported mean age of 25 to 30 years, ranging 11-73 years[2,3]. In the elderly population it has been suggested that SPTP tend to be malignant[4,5]. It was first described by Frantz in 1959[6]. Multiple descriptive names have been used for this tumor, including papillary epithelial neoplasm, papillary/cystic neoplasm, solid-and-papillary epithelial neoplasm, papillary-cystic carcinoma, solid-and papillary neoplasm, low-grade papillary neoplasm, and the Frantz tumor[7]. The WHO pancreatic tumor working group recently recommended the use of the term solid-pseudopapillary neoplasm[8], a term that has been widely used and accepted by pathologists and clinicians in daily practice[9,10].

Almost 610 SPTPs, including more than 57 diagnosed by percutaneous fine-needle aspiration (FNA), have been reported since the initial description in 1959[6]. In recent years, image-guided FNA has been increasingly performed for pancreatic lesions. Endoscopic ultrasound (EUS)-guided FNA can have an important role and provide an

accurate preoperative diagnosis particularly when the EUS findings of the mass are inconclusive. EUS-guided FNA can differentiate SPTP from other pancreatic neoplasms of similar radiologic and cytologic appearance but with different biologic behaviour and treatment, such as pancreatic endocrine tumors, acinar cell carcinoma, and papillary mucinous carcinoma[11-17]. The cytomorphology of this tumor is highly characteristic, with features that are distinctive from those of other cystic and solid tumors of the pancreas. It is important that this tumor is accurately diagnosed because management protocols differ from other tumor types originating in the pancreas.

In this paper, we describe the clinical, imaging, cytomorphologic features and differential diagnosis of a new case of SPTP diagnosed by EUS-guided FNA with a review of the literature.

CASE REPORT

A 17-year-old woman was admitted with complaints of an unexplained episodic pain for 2 mo and a short history of hypertension in the endocrinology clinic of our hospital (Athens General Hospital, Greece). Clinical laboratory examinations revealed polycystic ovary syndrome (PCOs), splenomegaly and low serum amylase and carcinoembryonic antigen (CEA) levels. CT-scan revealed a mass of the pancreatic tail with solid and cystic consistency.

EUS-guided FNA was performed using 22-gauge needles *via* a transgastric approach. Smears were made at the bedside in the endoscopy suite. The aspirated material was smeared onto glass slides, air-dried, and immediately stained with rapid Hemo-color stain for specimen adequacy assessment and preliminary diagnostic interpretation. Other smears also were fixed immediately in 95% alcohol for subsequent Papanicolaou staining. Additional aspirated material was fixed in formalin, embedded in paraffin, and processed for routine histologic examination using standard techniques. Staining with periodic acid Schiff (PAS) and Alcian-blue (AB) stains was performed. Immunohistochemical stains for vimentin (Dako; 1:100), neuron-specific enolase (NSE) (Dako; 1:100), synaptophysin (NovoCastra, Newcastle UK), chromogranin (Dako; 1:800), CA 19.9 (Dako), a1-antitrypsin (Dako; 1:3200), a1-antichymotrypsin (Dako; 1:800), were also performed. Avidin-biotin peroxidase complex technique was used.

Smears and cell block sections were examined with an emphasis on the evaluation of cytomorphologic features and immunohistochemical results.

EUS confirmed a mass, both in body and tail of the pancreas, with distinct borders, which caused dilation of the peripheral part of the pancreatic duct [major diameter (m.d.) 3.7 mm]. More specifically the tumor mass was solid and cystic, hypoechoic and heterogenous with a size measuring 65.4 mm × 54.2 mm (Figure 1). The EUS differential diagnosis included serous cystadenoma, mucinous cystadenoma, mucinous cystadenocarcinoma, adenocarcinoma with cystic degeneration, endocrine tumor and SPTP.

Smears were hypercellular and characteristically showed branching papillary arrangements composed of delicate fibrovascular cores and microadenoid structures with attached monotonous cuboidal neoplastic cells (Figure 2A-B). The nuclei of malignant cells were round or oval, eccentric with finely granular chromatin, small nucleoli and in some of them nuclear grooves. Cytoplasm was granular or finely vacuolated with wispy borders. No mitotic activity or significant atypia was observed. The architectural features were more evident in the cell block sections. Histochemically, tumor cells revealed PAS Alcian-blue (+) (Figure 3A-B). Immunostains performed on the cell block yielded vimentin positivity (Figure 4A), CA 19.9 positivity (Figure 4B), synaptophysin, a1-antitrypsin and a1-antichymotryspin focal positivity whereas NSE and chromogranin were negative. These findings were strongly suggestive of SPTP.

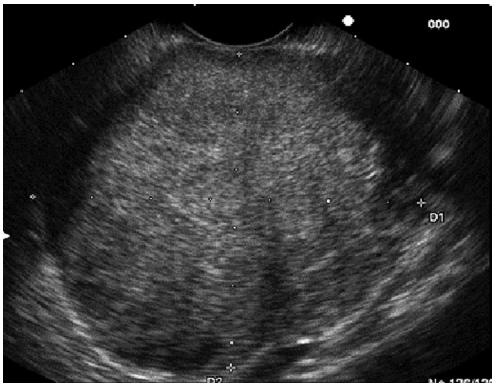


Figure 1 Endoscopic ultrasound image (EUS) showing a mass in body and tail of the pancreas.

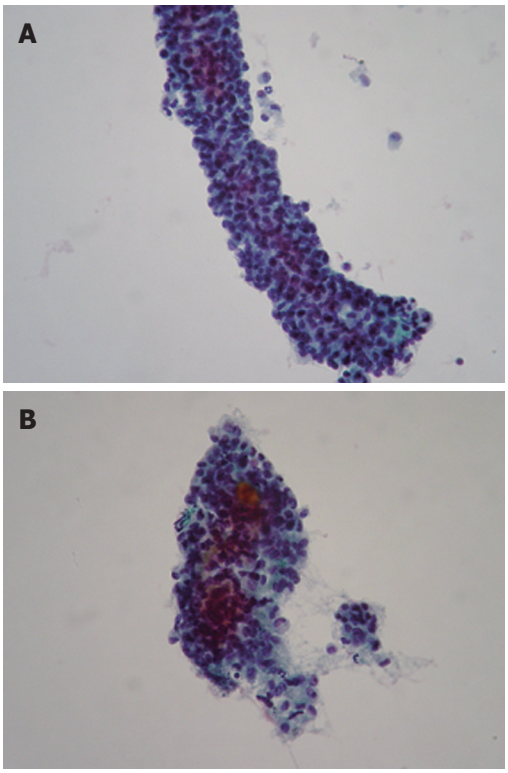


Figure 2 Papillary arrangement composed of delicate fibrovascular core with attached monotonous cuboidal neoplastic cells (**A**) and adenoid structures composed of cuboidal neoplastic cells (**B**) (PAP, x 400).

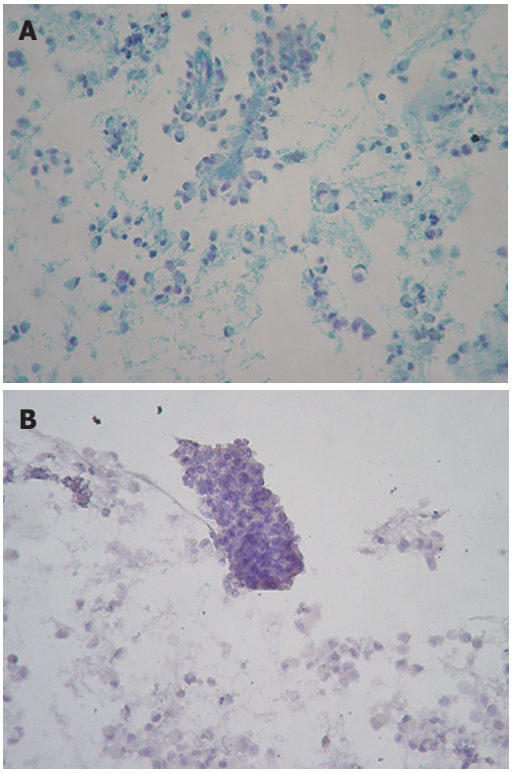


Figure 3 Staining with periodic acid Schiff (PAS) (**A**) and Alcian-blue (AB) (**B**) (x 400).

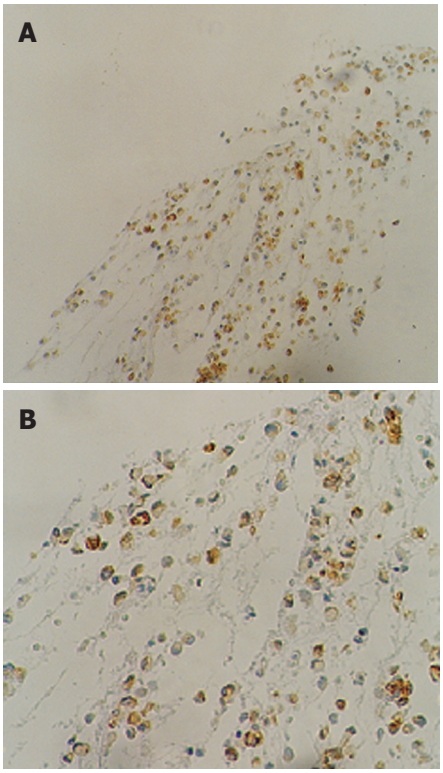


Figure 4 Immunostain for vimentin (**A**) and CA 19.9 (**B**) (x 400).

DISCUSSION

Solid-pseudopapillary neoplasm is an exceedingly rare pancreatic tumor with a reported frequency of less than 1% of all pancreatic diseases. Most of these reports are in the form of small case series with only few reports with greater than 50 cases identified in the literature.

It predominantely occurs in adolescent girls and young women with a reported frequency of 87% to 90% (mean age of 25 to 35 years)[2,3]. Such a striking predilection of SPTP occurring in women has suggested a hormonal influence on the pathogenesis of this tumor[18,19]. Cases occurring at the first decade of life are rare and less than 10% of SPTP cases have been reported in patients older than 40 years[2,20]. Occurrence of SPTP in men is rare, accounting for 7% of cases[21].

Clinically, SPTP may present as an abdominal mass with discomfort or pain, or it may be an incidental finding in work up for unrelated conditions. These tumors are generally large with a mean diameter of 10.3 cm and approximately 72% arise in the body and tail of the pancreas and less frequently in the head[2]. Unusual presentations of SPTP include multicentricity[22,23], occurrence in extrapancreatic sites such as mesocolon[24], retroperitoneum[25], omentum[26], liver[27] and duodenum[28]. In our case, the sex (female), age (17 years old) and tumor localization (body and tail of the pancreas) were typical of SPTP.

In most patients, the tumor follows an indolent clinical course and complete resection is often curative[20,29]. Up to 15% of cases have shown aggressive behaviour consisting of extension into adjacent blood vessels and organs, local recurrence and distant metastasis[20,28,30,31]. However, even metastatic SPTPs are growing slowly and have excellent prognosis. Only one death, due to metastatic spread of SPTP has been reported[32]. Also, spontaneously regressing tumors have been reported[33].

Benign-appearing SPTP might contrast with cellular anaplasia present in the metastatic deposits[34]. Thus, there are no histological features that can predict aggressive clinical behaviour. Proposed pathologic features related to aggressive behaviour or metastatic potential include diffuse growth pattern, venous invasion, nuclear pleomorphism, mitotic rate, necrosis and areas of dedifferentiation[35]. Most SPTPs display diploid DNA content[1]. However, DNA aneuploidy is more prominent in malignant SPTPs[34,36]. Chromosomal abnormalities, including double loss of X chromosomes and trisomy for chromosome 3, and unbalanced translocation between chromosomes 13 and 17 have been found in SPTP associated with aggressive behaviour and might be indicators of possible metastatic potential[37,38]. Thus, since the malignant potential of SPTP is difficult to determine, this tumor is considered a low malignant or borderline malignant potential.

The histogenesis of SPTP has been, and to date remains, elusive. Multiple theories have been proposed, including origin from small duct epithelium, derivation from acinar cells, endocrine pancreas, totipotential stem cells, or primitive cells capable of differentiating exocrine and endocrine[4,39-41], along with genital ridge-related cells that are incorporated in the pancreas during early embryogenesis[42]. Another recent report showed that SPTP is positive for melanocytic markers (S-100 protein, human melanoma black 45 (HMB45) and melanoma antigen recognized by T-cells 1 (MART-1) by immunohistochemistry and demonstrated the presence of premelanosomes and melanosome granules in the tumor cells by transmission electron microscopy, suggesting that this tumor may be of neural

crest origin[43]. Also, predilection in young women suggests that sex hormones play a role in the histogenesis or progression of this tumor. However, the ER and PR expression are not consistent in the literature. Using a binding essay, Ladanyi *et al*[18] demonstrated the expression of ER in SPTP and proposed that this tumor is hormone sensitive.

Diagnosis of SPTP is important for the clinical management of patients with this tumor. Diagnostic modalities including CT and magnetic resonance imaging can only suggest a diagnosis of SPTP. CT findings include an encapsulated lesion with well-defined borders and variable central areas with cystic degeneration, necrosis, or hemorrhage. Calcifications may occasionally be seen. Magnetic resonance imaging is helpful for identifying the characteristic internal signal intensities of blood products, which help distinguish this tumor from other cystic pancreatic tumors[44].

In recent years, advances in technology have permitted the performance of fine-needle aspiration biopsy under EUS guidance[45,46]. The overall accuracy of EUS is superior to CT scan and magnetic resonance imaging for detecting pancreatic lesions. It has been shown that EUS alone (94%) is more sensitive than CT scan (69%) and magnetic resonance imaging (83%) for detecting lesions, especially when they are smaller than 3.0 cm[47]. EUS permits a better evaluation of SPTPs, but the findings also are not specific. A small SPTP, often a monocystic tumor most frequent in males, might have the EUS appearance of a solid endocrine neoplasm, and it might be difficult to distinguish between the two[48].

An accurate preoperative diagnosis is highly desirable, since local surgical excision is usually curative and this is possible by EUS-guided FNA cytology. Bondeson *et al*[49] were the first to correctly diagnose SPTP by preoperative FNA.

Since then, to our knowledge, cytologic findings of percutaneous FNA-diagnosed SPTP have been described in 57 cases[9,21,22,31,37,49-53]. However, only few cases have been diagnosed by EUS-guided FNA[9,54]. FNA cytomorphologic features are highly characteristic and distinct from those of other cystic or solid tumors of the pancreas. On aspirated matrials, the most frequent features are the presence of marked cellularity with pseudopapillary fragments composed of fibrovascular stalks lined with one to several layers of tumor cells intermingled with discohesive neoplastic cells[1,21,22,49,51]. Careful evaluation of smears for mitotic activity is recommended, since this parameter has recently been considered one predictive criterion for biologic aggressiveness[55]. Inter or intracellular pink hyaline globules, mucus-like globules, as in our case, surrounded by stromal cells and cellular debris are also frequent features.

Immunohistochemically, most SPTPs are immunoreactive for vimentin (vim), a1-antitrypsin (AAT), a1-antichymotrypsin (AACT)[56], occassionally positive for neuron-specific enolase and synaptophysin (syn)[9], and nonreactive for S-100, CA 19.9 and chromogranin A. In our case, SPTP was vim (+), AAT (+), AACT (+), CA 19.9 (+) and syn (+). The above-mentioned cytologic and immunohistochemical features are strongly suggestive of SPTP.

The differential diagnosis of SPTP includes pancreatic neuroendocrine tumor, acinar-cell carcinoma, papillary mucinous carcinoma and intraductal papillary mucinous tumor. Pancreatic endocrine tumors predominantly occur in older patients and can be associated with a variety of clinical syndromes. These tumors share some cytological features with SPTP. The presence of rosettes without papillary structures and typical "salt and pepper" chromatin indicates a pancreatic endocrine tumor[20,16]. Additionally, these tumors express neuroendocrine markers such as chromogranin, neuron-specific enolase, and synaptophysin which are usually nonreactive in cases of SPTP. Acinar-cell carcinoma occurring in a wide range of age, is more commonly observed in males, and shows no predilection for head, body or tail location in the pancreas. Aspirates are cellular and composed only of acinar neoplastic cells containing enlarged nuclei with irregular membranes and distinct nucleoli. Patients with papillary mucinous carcinoma often have a single, large unilocular cystic mass and the neoplastic cells are columnar with cytoplasmic vacuoles, variable nuclear anaplasia, prominent nucleoli and mucinous background that should not be mistaken for the myxoid stroma[22,57]. Also, the thick, glistening and viscid mucus almost always present in intraductal papillary mucinous tumor is an important feature that distinguishes this neoplasm from SPTP[58].

In conclusion, we believe that EUS-FNA provides an excellent cellular yield and an overall sensitivity for the diagnosis of SPTP. Clinical correlation, radiological findings and cytomorphologic features from EUS-guided FNA achieve the accurate diagnosis of SPTP.

REFERENCES

1 **Pettinato G**, Manivel JC, Ravetto C, Terracciano LM, Gould EW, di Tuoro A, Jaszcz W, Albores-Saavedra J. Papillary cystic tumor of the pancreas. A clinicopathologic study of 20 cases with cytologic, immunohistochemical, ultrastructural, and flow cytometric observations, and a review of the literature. *Am J Clin Pathol* 1992; **98**: 478-488

2 **Kosmahl M**, Pauser U, Peters K, Sipos B, Lüttges J, Kremer B, Klöppel G. Cystic neoplasms of the pancreas and tumor-like lesions with cystic features: a review of 418 cases and a classification proposal. *Virchows Arch* 2004; **445**: 168-178

3 **Brázdil J**, Hermanová M, Kren L, Kala Z, Neumann C, Růzicka M, Nenutil R. Solid pseudopapillary tumor of the pancreas: 5 case reports. *Rozhl Chir* 2004; **83**: 73-88

4 **Matsunou H**, Konishi F. Papillary-cystic neoplasm of the pancreas. A clinicopathologic study concerning the tumor aging and malignancy of nine cases. *Cancer* 1990; **65**: 283-291

5 **Takahashi H**, Hashimoto K, Hayakawa H, Kusakawa M, Okamura K, Kosaka A, Mizumoto R, Katsuta K. Solid cystic tumor of the pancreas in elderly men: report of a case. *Surg Today* 1999; **29**: 1264-1267

6 **Frantz VK**. Tumor of the pancreas. In: Atlas of Tumor Pathology: Fascicles 22 and 28, Section 7. Washington DC: US Armed Forces Institute of Pathology, 1959: 32-33

7 **Zhou H**, Cheng W, Lam KY, Chan GC, Khong PL, Tam PK. Solid-cystic papillary tumor of the pancreas in children. *Pediatr Surg Int* 2001; **17**: 614-620

8 **Klöppel G**, Luttges J, Klimstra D. Solid-pseudopapillary neoplasm. In: Hamilton SR, Aaltonen LA, editors. WHO Classification of Tumors Pathology and Genetics, Tumor of the Digestive System. Lyon, France: IARC, 2000: 246-248

9 **Bardales RH**, Centeno B, Mallery JS, Lai R, Pochapin M, Guiter G, Stanley MW. Endoscopic ultrasound-guided fine-needle aspiration cytology diagnosis of solid-pseudopapillary

tumor of the pancreas: a rare neoplasm of elusive origin but characteristic cytomorphologic features. *Am J Clin Pathol* 2004; **121**: 654-662
10 **Lack EE**. Solid-pseudopapillary neoplasma. In: Lack EE, editor. Pathology of the Pancreas, Gallbladder, Extrahepatic Biliary Tract, and Ampullary Region. New York: Oxford University Press, 2003: 281-290
11 **Koito K**, Namieno T, Nagakawa T, Shyonai T, Hirokawa N, Morita K. Solitary cystic tumor of the pancreas: EUS-pathologic correlation. *Gastrointest Endosc* 1997; **45**: 268-276
12 **Brugge WR**. Role of endoscopic ultrasound in the diagnosis of cystic lesions of the pancreas. *Pancreatology* 2001; **1**: 637-640
13 **Das DK**, Bhambhani S, Kumar N, Chachra KL, Prakash S, Gupta RK, Tripathi RP. Ultrasound guided percutaneous fine needle aspiration cytology of pancreas: a review of 61 cases. *Trop Gastroenterol* 1995; **16**: 101-109
14 **Nadler EP**, Novikov A, Landzberg BR, Pochapin MB, Centeno B, Fahey TJ, Spigland N. The use of endoscopic ultrasound in the diagnosis of solid pseudopapillary tumors of the pancreas in children. *J Pediatr Surg* 2002; **37**: 1370-1373
15 **Centeno BA**. Fine needle aspiration biopsy of the pancreas. *Clin Lab Med* 1998; **18**: 401-427, v-vi
16 **Collins BT**, Saeed ZA. Fine needle aspiration biopsy of pancreatic endocrine neoplasms by endoscopic ultrasonographic guidance. *Acta Cytol* 2001; **45**: 905-907
17 **Rampy BA**, Waxman I, Xiao SY, Logroño R. Serous cystadenoma of the pancreas with papillary features: a diagnostic pitfall on fine-needle aspiration biopsy. *Arch Pathol Lab Med* 2001; **125**: 1591-1594
18 **Ladanyi M**, Mulay S, Arseneau J, Bettez P. Estrogen and progesterone receptor determination in the papillary cystic neoplasm of the pancreas. With immunohistochemical and ultrastructural observations. *Cancer* 1987; **60**: 1604-1611
19 **Wrba F**, Chott A, Ludvik B, Schratter M, Spona J, Reiner A, Schernthaner G, Krisch K. Solid and cystic tumour of the pancreas; a hormonal-dependent neoplasm? *Histopathology* 1988; **12**: 338-340
20 **Lam KY**, Lo CY, Fan ST. Pancreatic solid-cystic-papillary tumor: clinicopathologic features in eight patients from Hong Kong and review of the literature. *World J Surg* 1999; **23**: 1045-1050
21 **Pettinato G**, Di Vizio D, Manivel JC, Pambuccian SE, Somma P, Insabato L. Solid-pseudopapillary tumor of the pancreas: a neoplasm with distinct and highly characteristic cytological features. *Diagn Cytopathol* 2002; **27**: 325-334
22 **Young NA**, Villani MA, Khoury P, Naryshkin S. Differential diagnosis of cystic neoplasms of the pancreas by fine-needle aspiration. *Arch Pathol Lab Med* 1991; **115**: 571-577
23 **Orlando CA**, Bowman RL, Loose JH. Multicentric papillary-cystic neoplasm of the pancreas. *Arch Pathol Lab Med* 1991; **115**: 958-960
24 **Tornóczky T**, Kálmán E, Jáksó P, Méhes G, Pajor L, Kajtár GG, Battyány I, Davidovics S, Sohail M, Krausz T. Solid and papillary epithelial neoplasm arising in heterotopic pancreatic tissue of the mesocolon. *J Clin Pathol* 2001; **54**: 241-245
25 **Klöppel G**, Maurer R, Hofmann E, Lüthold K, Oscarson J, Forsby N, Ihse I, Ljungberg O, Heitz PU. Solid-cystic (papillary-cystic) tumours within and outside the pancreas in men: report of two patients. *Virchows Arch A Pathol Anat Histopathol* 1991; **418**: 179-183
26 **Fukunaga M**. Pseudopapillary solid cystic tumor arising from an extrapancreatic site. *Arch Pathol Lab Med* 2001; **125**: 1368-1371
27 **Kim YI**, Kim ST, Lee GK, Choi BI. Papillary cystic tumor of the liver. A case report with ultrastructural observation. *Cancer* 1990; **65**: 2740-2746
28 **Pasquiou C**, Scoazec JY, Gentil-Perret A, Taniere P, Ranchere-Vince D, Partensky C, Barth X, Valette PJ, Bailly C, Mosnier JF, Berger F. Solid pseudopapillary tumors of the pancreas. Pathology report of 13 cases. *Gastroenterol Clin Biol* 1999; **23**: 207-214
29 **Mao C**, Guvendi M, Domenico DR, Kim K, Thomford NR, Howard JM. Papillary cystic and solid tumors of the pancreas: a pancreatic embryonic tumor? Studies of three cases and cumulative review of the world's literature. *Surgery* 1995; **118**: 821-828
30 **González-Cámpora R**, Rios Martin JJ, Villar Rodriguez JL, Otal Salaverri C, Hevia Vazquez A, Valladolid JM, Portillo M, Galera Davidson H. Papillary cystic neoplasm of the pancreas with liver metastasis coexisting with thyroid papillary carcinoma. *Arch Pathol Lab Med* 1995; **119**: 268-273
31 **Compagno J**, Oertel JE, Krezmar M. Solid and papillary epithelial neoplasm of the pancreas, probably of small duct origin: a clinicopathologic study of 52 cases. *Lab Invest* 1979; **40**: 248-249
32 **Buetow PC**, Buck JL, Pantongrag-Brown L, Beck KG, Ros PR, Adair CF. Solid and papillary epithelial neoplasm of the pancreas: imaging-pathologic correlation on 56 cases. *Radiology* 1996; **199**: 707-711
33 **Hachiya M**, Hachiya Y, Mitsui K, Tsukimoto I, Watanabe K, Fujisawa T. Solid, cystic and vanishing tumors of the pancreas. *Clin Imaging* 2003; **27**: 106-108
34 **Cappellari JO**, Geisinger KR, Albertson DA, Wolfman NT, Kute TE. Malignant papillary cystic tumor of the pancreas. *Cancer* 1990; **66**: 193-198
35 **Washington K**. Solid-pseudopapillary tumor of the pancreas: challenges presented by an unusual pancreatic neoplasm. *Ann Surg Oncol* 2002; **9**: 3-4
36 **Kamei K**, Funabiki T, Ochiai M, Amano H, Marugami Y, Kasahara M, Sakamoto T. Some considerations on the biology of pancreatic serous cystadenoma. *Int J Pancreatol* 1992; **11**: 97-104
37 **Grant LD**, Lauwers GY, Meloni AM, Stone JF, Betz JL, Vogel S, Sandberg AA. Unbalanced chromosomal translocation, der (17) t (13;17) (q14;p11) in a solid and cystic papillary epithelial neoplasm of the pancreas. *Am J Surg Pathol* 1996; **20**: 339-345
38 **Matsubara K**, Nigami H, Harigaya H, Baba K. Chromosome abnormality in solid and cystic tumor of the pancreas. *Am J Gastroenterol* 1997; **92**: 1219-1221
39 **Stömmer P**, Kraus J, Stolte M, Giedl J. Solid and cystic pancreatic tumors. Clinical, histochemical, and electron microscopic features in ten cases. *Cancer* 1991; **67**: 1635-1641
40 **Yagihashi S**, Sato I, Kaimori M, Matsumoto J, Nagai K. Papillary and cystic tumor of the pancreas. Two cases indistinguishable from islet cell tumor. *Cancer* 1988; **61**: 1241-1247
41 **Miettinen M**, Partanen S, Fräki O, Kivilaakso E. Papillary cystic tumor of the pancreas. An analysis of cellular differentiation by electron microscopy and immunohistochemistry. *Am J Surg Pathol* 1987; **11**: 855-865
42 **Kosmahl M**, Seada LS, Jänig U, Harms D, Klöppel G. Solid-pseudopapillary tumor of the pancreas: its origin revisited. *Virchows Arch* 2000; **436**: 473-480
43 **Chen C**, Jing W, Gulati P, Vargas H, French SW. Melanocytic differentiation in a solid pseudopapillary tumor of the pancreas. *J Gastroenterol* 2004; **39**: 579-583
44 **Cantisani V**, Mortele KJ, Levy A, Glickman JN, Ricci P, Passariello R, Ros PR, Silverman SG. MR imaging features of solid pseudopapillary tumor of the pancreas in adult and pediatric patients. *AJR Am J Roentgenol* 2003; **181**: 395-401
45 **Wiersema MJ**, Hawes RH, Tao LC, Wiersema LM, Kopecky KK, Rex DK, Kumar S, Lehman GA. Endoscopic ultrasonography as an adjunct to fine needle aspiration cytology of the upper and lower gastrointestinal tract. *Gastrointest Endosc* 1992; **38**: 35-39
46 **Vilmann P**, Jacobsen GK, Henriksen FW, Hancke S. Endoscopic ultrasonography with guided fine needle aspiration biopsy in pancreatic disease. *Gastrointest Endosc* 1992; **38**: 172-173
47 **Müller MF**, Meyenberger C, Bertschinger P, Schaer R, Marincek B. Pancreatic tumors: evaluation with endoscopic US, CT, and MR imaging. *Radiology* 1994; **190**: 745-751
48 **Uchimi K**, Fujita N, Noda Y, Kobayashi G, Kimura K, Matsunaga A, Yuki T, Nomura M, Sato T, Ishida K, Seno S, Ito K, Okubo K, Suzuki T, Hirasawa D, Sugawara T, Horaguchi J, Tada T, Takazawa O. Solid cystic tumor of the pancreas: report of six cases and a review of the Japanese literature. *J*

Gastroenterol 2002; **37**: 972-980
49 **Bondeson L**, Bondeson AG, Genell S, Lindholm K, Thorstenson S. Aspiration cytology of a rare solid and papillary epithelial neoplasm of the pancreas. Light and electron microscopic study of a case. *Acta Cytol* 1984; **28**: 605-609
50 **Pelosi G**, Iannucci A, Zamboni G, Bresaola E, Iacono C, Serio G. Solid and cystic papillary neoplasm of the pancreas: a clinico-cytopathologic and immunocytochemical study of five new cases diagnosed by fine-needle aspiration cytology and a review of the literature. *Diagn Cytopathol* 1995; **13**: 233-246
51 **Katz LB**, Ehya H. Aspiration cytology of papillary cystic neoplasm of the pancreas. *Am J Clin Pathol* 1990; **94**: 328-333
52 **Granter SR**, DiNisco S, Granados R. Cytologic diagnosis of papillary cystic neoplasm of the pancreas. *Diagn Cytopathol* 1995; **12**: 313-319
53 **Bhanot P**, Nealon WH, Walser EM, Bhutani MS, Tang WW, Logroño R. Clinical, imaging, and cytopathological features of solid pseudopapillary tumor of the pancreas: a clinicopathologic study of three cases and review of the literature. *Diagn Cytopathol* 2005; **33**: 421-428
54 **Mergener K**, Detweiler SE, Traverso LW. Solid pseudopapillary tumor of the pancreas: diagnosis by EUS-guided fine-needle aspiration. *Endoscopy* 2003; **35**: 1083-1084
55 **Nishihara K**, Nagoshi M, Tsuneyoshi M, Yamaguchi K, Hayashi I. Papillary cystic tumors of the pancreas. Assessment of their malignant potential. *Cancer* 1993; **71**: 82-92
56 **Liu X**, Rauch TM, Siegal GP, Jhala N. Solid-pseudopapillary neoplasm of the pancreas: Three cases with a literature review. *Appl Immunohistochem Mol Morphol* 2006; **14**: 445-453
57 **Naresh KN**, Borges AM, Chinoy RF, Soman CS, Krishnamurthy SC. Solid and papillary epithelial neoplasm of the pancreas. Diagnosis by fine needle aspiration cytology in four cases. *Acta Cytol* 1995; **39**: 489-493
58 **Stelow EB**, Stanley MW, Bardales RH, Mallery S, Lai R, Linzie BM, Pambuccian SE. Intraductal papillary-mucinous neoplasm of the pancreas. The findings and limitations of cytologic samples obtained by endoscopic ultrasound-guided fine-needle aspiration. *Am J Clin Pathol* 2003; **120**: 398-404

S- Editor Zhu LH **L- Editor** Wang XL **E- Editor** Li JL

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5164
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 published in this issue and those rejected for this issue) during the last editing time period.

Ibrahim Abdulkarim Al Mofleh, Professor
Deaprtment of Medicine, College of Medicine, King Saud University, P.O. Box 2925, Riyadh 11461, Saudi Arabia

Akira Andoh, MD
Department of Internal Medicine, Shiga University of Medical Science, Seta Tukinowa, Otsu 520-2192, Japan

Qasim Aziz, Professor
Gastroenterology Department of Gastrointestinal Science, Hope Hospital, Salford, M6 8HD, United Kindom

Marc Basson, MD, PhD, MBA, Chief of Surgery
John D. Dingell VA Medical Center, 4646 John R. Street, Detroit, MI 48301, United States

Trond Berg, Professor
Department of Molecular Biosciences, University of Oslo, PO Box 1041 Blindern, Oslo 0316, Norway

Markus W Büchler, MD
Department of General Surgery, University of Heidelberg, Im Neuenheimer Feld 110, Heidelberg D-69120, Germany

Yogesh K Chawla, Dr, Professor
Department of Hepatology, Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Giuseppe Chiarioni, Dr
Gastroenterological Rehabilitation Division of the University of Verona, Valeggio sul Mincio Hospital, Azienda Ospedale di Valeggio s/M, Valeggio s/M 37067, Italy

Paolo Del Poggio, Dr
Hepatology Unit Department of Internal Medicine, Treviglio Hospital, Piazza Ospedale 1, Treviglio Bg 24047, Italy

Chris E Forsmark, Professor
Division of Gastroenterology, Hepatology, and Nutrition , University of Florida, Box 100214, Room HD-602 1600 SW Archer Road Gainesville, FL, 32610-0214, United States

Nikolaus Gassler, Professor
Institute of Pathology, University Hospital RWTH Aachen, Pauwelsstrasse 30, 52074 Aachen, Germany

Shunji Ishihara, MD
Department of Gastroenterology and Hepatology, Shimane University, School of Medicine, 89-1, Enya-cho, Izumo 693-8501, Japan

Leonard R Johnson, Professor
Department of Physiology, University Tennessee College of Medicine, 894 Union Ave, Memphis, TN 38163, United States

Khandoga Andrej Khandoga, MD
Institute for Surgical Research Ludwig-Maximilians-University of Munich, Marchioninistr. 27, 81377 Munich, Germany

Ioannis E Koutroubakis, Assistant Professor
Ioannis Koutroubakis, Gastroenterology, University Hospital Heraklion, P.O.BOX:1352 Heraklion, 71110 Crete, Greece

Leonidas G Koniaris, Professor
Alan Livingstone Chair in Surgical Oncology, 3550 Sylvester Comprehensive Cancer Center (310T), 1475 NW 12th Ave., Miami, FL 33136, United States

Shiu-Ming Kuo, MD
University at Buffalo, 15 Farber Hall, 3435 Main Street, Buffalo 14214, United States

Kurt Lenz, Professor
Department of Internal Medicine, Konventhospital Barmherzige Brueder, A-4020 Linz, Austria

Stuart AC McDonald
Histopathology Unit, Cancer Research UK, Rm 337 3rd Floor, WC2A 3PX, London, United Kingdom

Sabine Mihm, Professor
Department of Gastroenterology, Georg-August-Universität, Robert-Koch-Str.40, Göttingen D-37099, Germany

James Michael Millis, Professor
University of Chicago, Section of Transplantation, MC 5027, 5841 S. Maryland Avenue, Chicago, IL 60637, United States

Gerardo Nardone, MD, Associate Professor
Department of Clinical and Experimental Medicine, University of Naples Federico II, Via Pansini 5, Napoli 80131, Italy

Satoshi Osawa, MD
First Department of Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu, 431-3192, Japan

Thierry Piche, MD, PhD
Department of Gastroenterology, Archet 2 Hospital, 151 RTE ST Antoine de Ginestiere 06202, Nice CEDEX 3, France

Richard A Rippe, Dr
Department of Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7038, United States

Massimo Raimondo
Dr Division of Gastroenterology and Hepatology, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, United States

Luis Rodrigo, Professor
Gastroenterology Service, Hospital Central de Asturias, c/Celestino Villamil, s.n., Oviedo 33.006, Spain

Riina Salupere, MD, PhD
Division of Endocrinology and Gastroenterology, University of Tartu, L.Puusepa street 6, Tartu 51014, Estonia

Tomohiko Shimatani
Assistant Professor, Department of General Medicine, Hiroshima University Hospital, 1-2-3 Kasumi, Minami-ku, Hiroshima 7348551, Japan

Andrew Ukleja, MD, Assistant Professor
Clinical Assistant Professor of Medicine, Director of Nutrition Support Team, Director of Esophageal Motility Laboratory, Cleveland Clinic Florida, Department of Gastroenterology, 2950 Cleveland Clinic Blvd., Weston, FL 33331, United States

Fritz von Weizsäcker, Professor
Department of Medicine Schlosspark-Klinik, Humboldt University, Heubnerweg 2, Berlin D-14059, Germany

Jens Werner, MD, Associate Professor
Department of General and Visceral Surgery, University of Heidelberg, INF 110, Heidelberg 69120, Germany

James YW Lau
Department of Surgery, Prince of Wales Hospital, the Chinese University of Hong Kong, Hong Kong, China

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5165
World Journal of Gastroenterology ISSN 1007-9327

Meetings

MAJOR MEETINGS COMING UP

Meeting Falk Research Workshop: Morphogenesis and Cancerogenesis of the Liver
25-26 January 2007
Goettingen
symposia@falkfoundation.de

Meeting Canadian Digestive Diseases Week (CDDW)
16-20 February 2007
Banff-AB
cagoffice@cag-acg.org
www.cag-acg.org/cddw/cddw2007.htm

Meeting Falk Symposium 158: Intestinal Inflammation and Colorectal Cancer
23-24 March 2007
Sevilla
symposia@falkfoundation.de

Meeting BSG Annual Meeting
26-29 March 2007
Glasgow
www.bsg.org.uk/

NEXT 6 MONTHS

Meeting 42nd Annual Meeting of the European Association for the Study of the Liver
11-15 April 2007
Barcelona
easl2007@easl.ch
www.easl.ch/liver-meeting/

Meeting Falk Symposium 159: IBD 2007 - Achievements in Research and Clinical Practice
4-5 May 2007
Istanbul
symposia@falkfoundation.de

Meeting European Society for Paediatric Gastroenterology, Hepatology and Nutrition Congress 2007
9-12 May 2007
Barcelona
espghan2007@colloquium.fr

Digestive Disease Week
19-24 May 2007
Washington Convention Center, Washington DC

Meeting Gastrointestinal Endoscopy Best Practices: Today and Tomorrow, ASGE Annual Postgraduate Course at DDW
23-24 May 2007
Washington-DC
tkoral@asge.org

Meeting ESGAR 2007 18th Annual Meeting and Postgraduate Course
12-15 June 2007
Lisbon
fca@netvisao.pt

Meeting Falk Symposium 160: Pathogenesis and Clinical Practice in Gastroenterology
15-16 June 2007
Portoroz
symposia@falkfoundation.de

Meeting ILTS 13th Annual International Congress
20-23 June 2007
Rio De Janeiro
www.ilts.org

Meeting 9th World Congress on Gastrointestinal Cancer
27-30 June 2007
Barcelona
meetings@imedex.com

EVENTS AND MEETINGS IN 2007

Meeting Falk Research Workshop: Morphogenesis and Cancerogenesis of the Liver
25-26 January 2007
Goettingen
symposia@falkfoundation.de

Meeting Canadian Digestive Diseases Week (CDDW)
16-20 February 2007
Banff-AB
cagoffice@cag-acg.org
www.cag-acg.org/cddw/cddw2007.htm

Meeting Falk Symposium 158: Intestinal Inflammation and Colorectal Cancer
23-24 March 2007
Sevilla
symposia@falkfoundation.de

Meeting BSG Annual Meeting
26-29 March 2007
Glasgow
www.bsg.org.uk/

Meeting 42nd Annual Meeting of the European Association for the Study of the Liver
11-15 April 2007
Barcelona
easl2007@easl.ch
www.easl.ch/liver-meeting/

Meeting Falk Symposium 159: IBD 2007 - Achievements in Research and Clinical Practice
4-5 May 2007
Istanbul
symposia@falkfoundation.de

Meeting European Society for Paediatric Gastroenterology, Hepatology and Nutrition Congress 2007
9-12 May 2007
Barcelona
espghan2007@colloquium.fr

Meeting Gastrointestinal Endoscopy Best Practices: Today and Tomorrow, ASGE Annual Postgraduate Course at DDW
23-24 May 2007
Washington-DC
tkoral@asge.org

Meeting ESGAR 2007 18th Annual Meeting and Postgraduate Course
12-15 June 2007
Lisbon
fca@netvisao.pt

Meeting Falk Symposium 160: Pathogenesis and Clinical Practice in Gastroenterology
15-16 June 2007
Portoroz
symposia@falkfoundation.de

Meeting ILTS 13th Annual International Congress
20-23 June 2007
Rio De Janeiro
www.ilts.org

Meeting 9th World Congress on Gastrointestinal Cancer
27-30 June 2007
Barcelona
meetings@imedex.com

Meeting 15th International Congress of the European Association for Endoscopic Surgery
4-7 July 2007
Athens
info@eaes-eur.org
congresses.eaes-eur.org/

Meeting 39th Meeting of the European Pancreatic Club
4-7 July 2007
Newcastle
www.e-p-c2007.com

Meeting XXth International Workshop on Heliobacter and related bacteria in cronic degistive inflammation
20-22 September 2007
Istanbul
www.heliobacter.org

Meeting Falk Workshop: Mechanisms of Intestinal Inflammation
10 October 2007
Dresden
symposia@falkfoundation.de

Meeting Falk Symposium 161: Future Perspectives in Gastroenterology
11-12 October 2007
Dresden
symposia@falkfoundation.de

Meeting Falk Symposium 162: Liver Cirrhosis - From Pathophysiology to Disease Management
13-14 October 2007
Dresden
symposia@falkfoundation.de

American College of Gastroenterology Annual Scientific Meeting
12-17 October 2007
Pennsylvania Convention Center
Philadelphia, PA

Meeting APDW 2007 - Asian Pacific Digestive Disease Week 2007
15-18 October 2007
Kobe
apdw@convention.co.jp
www.apdw2007.org

15th United European Gastroenterology Week, UEGW
27-31 October 2007
Le Palais des Congrès de Paris, Paris, France

Meeting The Liver Meeting® 2007 - 57th Annual Meeting of the American Association for the Study of Liver Diseases
2-6 November 2007
Boston-MA
www.aasld.org

Gastro 2009, World Congress of Gastroenterology and Endoscopy London, United Kingdom 2009

Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2007 October 14; 13(38): 5166-5168
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, Science Citation Index Expanded (also known as SciSearch®) and Journal Citation Reports/Science Edition, *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 submissions

Online submissions are 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: +86-10-85381892
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 350 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 5-10 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 × 85 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 ^1F, ^2F, ^3F; 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, 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.wjgnet.com/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, p (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.