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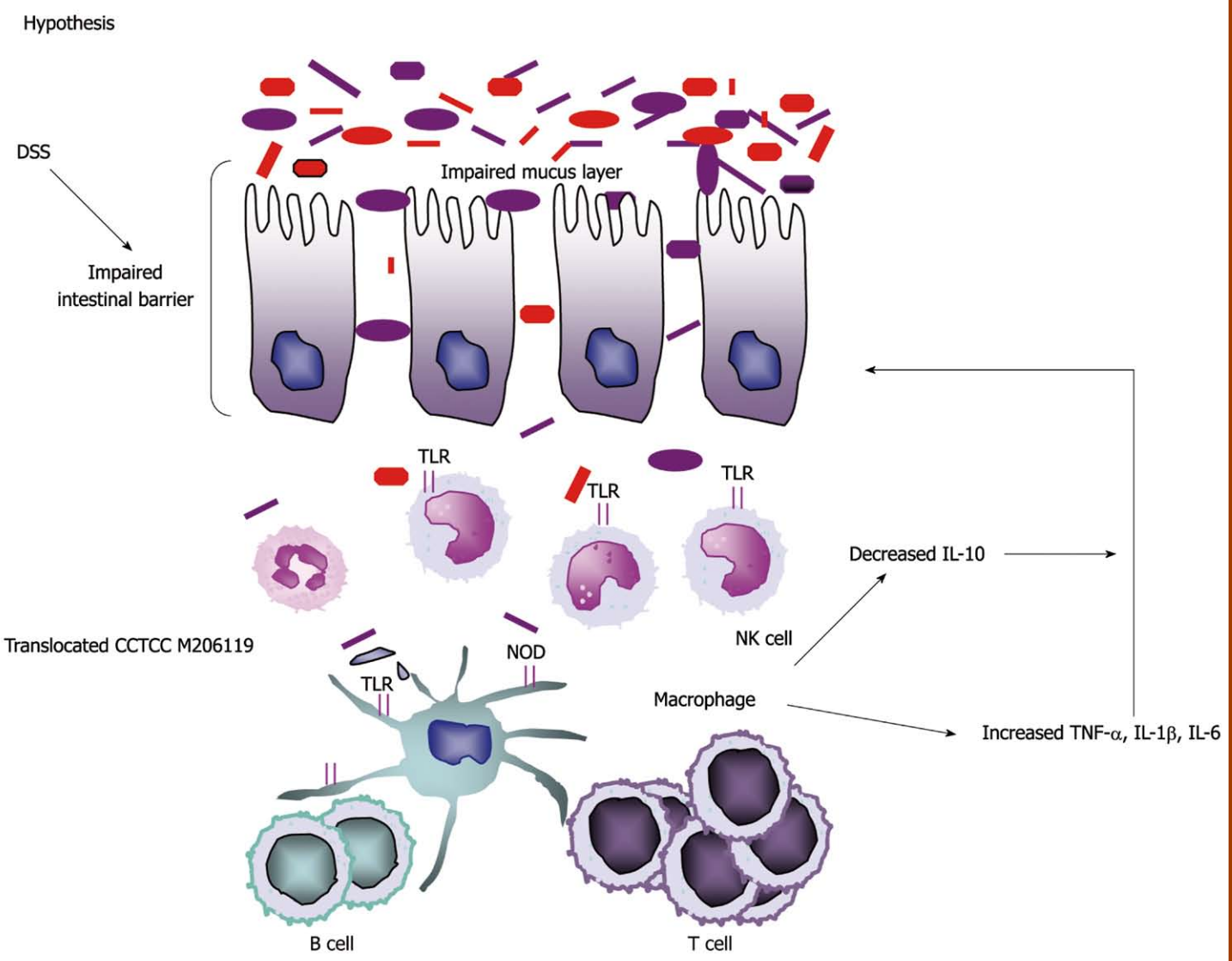

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EDITORIAL

Hepatitis C virus infection and health-related quality of life

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Abstract

Hepatitis C virus (HCV) hepatitis and other diseases related to HCV, such as cryoglobulinemia, lymphoma and renal failure, impair health-related quality of life (HRQoL). In addition, HCV *per se* might directly influence HRQoL *via* colonization of microglia in the brain or, indirectly, *via* the effect of systemic inflammatory cytokines which, in turn, can trigger brain interleukin production. The treatment of HCV-related disorders with interferon (IFN) has an effect on HRQoL. Initially, IFN causes a transient deterioration of HRQoL, due to the induction of depression and other side effects of treatment. Subsequently, the subjects who obtain a sustained virologic response experience an improvement in HRQoL. Only rarely does interferon treatment causes permanent detrimental effects on HRQoL, due to residual psychiatric or neurologic side effects. Liver transplantation is the only treatment for end-stage HCV-related liver disease. HRQoL generally improves massively a few months after transplantation, except in the case of serious complications of the transplant procedure. Furthermore, high levels of anxiety and neuroticism pre-transplant are associated with lower HRQoL one year after transplant. Additionally, six months after transplant, patients with HCV who experience virologic recurrence show significantly greater depression, anxiety, phobic anxiety, and paranoid ideation than anti-HCV-negative patients. In conclusion, optimal care for the overall well-being of patients with HCV infection requires adequate knowledge of their neurological and psychological status.

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Key words: Hepatitis C virus; Quality of life; Transplantation; Hepatitis; Cirrhosis

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INTRODUCTION

The concept of quality of life (QoL) is an attempt to define, in analyzable terms, the effect of functional outcome of a disease and its treatment on the patient. What emerges should be a functional definition that is measurable over time. The questions that are included in the evaluation of QoL are drawn from various domains of the experience of the patient and should, therefore, represent in the most complete way his/her self-perception concerning physical, psychological, relational and working experience. This conceptualization is based on the World Health Organization definition of health as "A state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity". This definition is so broad that it includes elements which are beyond the traditional domain of medicine and health

caring systems. Opportunity, education, spiritual attitudes, social security, working satisfaction, social relationships, goods availability are elements of QoL that are independent of medicine. What we deal with, by using the concept of health-related quality of life (HRQoL), is the functional effect of an illness and of its therapy on an individual.

Four broad domains contribute to HRQoL: (1) somatic sensations; (2) psychological state; (3) social interactions; and (4) physical and occupational functions.

Various questionnaires, if possible self-administered, have been developed to assess HRQoL. They are subdivided into generic tools, which encompass a global overview of the above-mentioned domains, and specific tools which are oriented to the peculiar consequences of a disease or a class of diseases.

The interest of HRQoL assessment in relation to hepatitis C virus (HCV) infection depends firstly on the acknowledgment that HCV infection is a major public health problem. It has been estimated that about 3% of humans in the word are infected by the HCV[1]. In Europe, it is estimated that there are approximately 5 million HCV carriers; the peak prevalence among individuals is in the fifth decade of age[2]. Therefore, HCV infection mainly involves adults in the period of their active life.

About 10%-20% of the individuals who are chronically infected by HCV are likely to develop liver cirrhosis over a median period of about 30 years[3] and, out of the HCV-infected patients with liver cirrhosis, 2%-6% per year develop hepatocellular carcinoma[4]. In addition, a minority of subjects will develop immunological disorders, such as cryoglobulinemia and lymphoma[5]. Obviously all these individuals will suffer from symptoms due to the diseases caused by HCV infection (but that can also have other causes), rather than from the virus infection *per se*.

Decompensated liver cirrhosis, the final stage of cirrhosis, has an ominous consequence both on survival[6,7] and on HRQoL[8]. This group of subjects display obvious limitations to HRQoL that are mainly related to the consequences of liver insufficiency and portal hypertension (i.e., hepatic encephalopathy, ascites, upper intestinal tract bleeding, sexual dysfunction, lower leg cramps and itch)[8]. However, the number of subjects with these complications of end-stage liver disease is relatively small, compared with the vast majority of patients having HCV in the absence of clinically significant liver disease.

Despite the initial idea that the majority of patients with HCV infection have merely asymptomatic seropositivity, some data suggest that HCV itself may diminish HRQoL even in the absence of advanced liver disease, perhaps due to HCV brain colonization or to systemic activation of cytokines which, in turn, influence brain cytokine production and neurotransmission.

Other important issues concerning HRQoL in HCV-infected patients are those related to the side effects of interferon (IFN) treatment and to liver transplantation, which is the treatment of choice of end-stage liver disease (Figure 1).

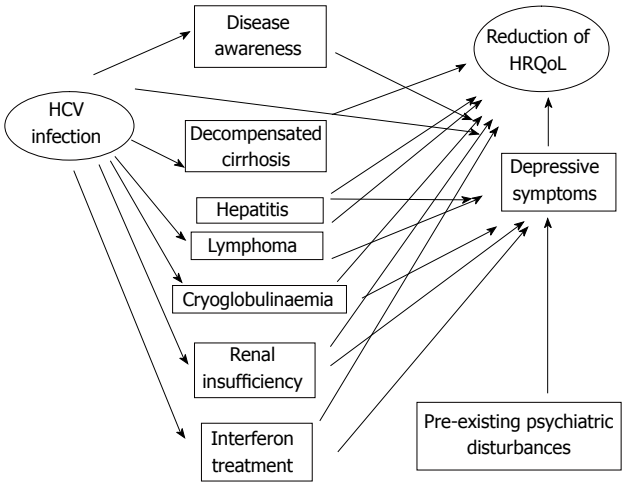

Figure 1 Pathways producing a reduction of health-related quality of life in hepatitis C virus-infected patients: both direct and indirect mechanisms play a role. HCV: Hepatitis C virus; HRQoL: Health-related quality of life.

HCV INFECTION *PER SE*

The issue of whether HCV infection *per se* (i.e., independent of the development of liver disease or other severe organic diseases related to HCV infection) may have an effect of HRQoL is a focus of debate. Several studies reported that patients with chronic HCV have a significant reduction in their HRQoL that is not related to the severity of the liver disease[9].

Depression can account for a reduction of HRQoL, since HRQoL depends on a patient's self-perception and evaluation. Therefore, any change in mood can obviously influence HRQoL. The prevalence of depression in HCV-infected individuals was reported to be higher than that in the general population (59% *vs* 21%)[10]. It should be noted, however, that depressive symptoms in patients with HCV infection might be due to pre-existing psychiatric disorders, to a reaction to the awareness of being infected, to adverse biological effects of HCV and, finally, to the consequences of liver disease; at least in the individuals who develop severe chronic hepatitis. In addition, coping styles and sources of information are of paramount importance in perceiving disease severity and disability, since they influence patients' stress levels and/or a pessimistic/optimistic view about the future[11].

An association between HCV infection and depression is therefore likely. However, the role of confounders-such as intravenous substance abuse or promiscuous sexual behaviour-related both to HCV infection and possibly to mood and psychological dimensions may bias statistical assessment. Therefore, further studies are required to determine with certainty the existence of a true association between depression and chronic HCV infection, together with its extent and the mechanisms implicated.

Moreover, strong clues for the existence of a true pathophysiological link between HCV infection and depression derive from the observation that depression has a higher prevalence in drug addicts who are infected with

HCV compared to those who are not infected[12]. In addition, HCV-infected patients who report no history of intravenous drug abuse complain of lower HRQoL compared with healthy normal subjects[13].

As mentioned above, the most difficult confounder to be controlled is the effect of the patient's awareness of his/her infection. In fact, it is reasonable to assume that the awareness of being infected with a virus which potentially is both life-threatening and transmittable by sexual intercourse may have an adverse emotional affect *per se*, regardless of any biological effect of the virus. However, the observation that patients with chronic HCV infection have reduced HRQoL scores compared to those with hepatitis B virus infection, suggests that HCV infection *per se* reduces HRQoL, rather than the non-specific patient awareness of a viral infection[13]. Also, HRQoL was found to be reduced even in patients who were not aware of being HCV carriers[14].

Some pathophysiological insight into the possible mechanisms involved in the reduction of HRQoL due to HCV reduction was provided by Weissenborn *et al*[15] who reported alterations of mood (increased anxiety and depression) and cognition, together with changes in both the midbrain serotoninergic and striatal dopaminergic systems, irrespective of viraemia and normal liver function, in patients with HCV infection. The existence of brain alteration directly caused by HCV is provided by the evidence of deficits of attention, executive function and verbal learning, of electroencephalogram slowing in the absence of liver cirrhosis and/or substance use disorder, and of peculiar alterations on magnetic resonance spectroscopy in HCV-infected patients[16-18]. It has been suggested that monocytes infected by HCV can cross the blood-brain barrier producing a secondary infection of microglia[19-22]. Such an infection is thought to be facilitated by immunosuppression caused by human immunodeficiency virus or by immunosuppressive treatment in transplanted patients[17,21-23].

Microglia infection by HCV may disturb neuronal function due to local cytokine production[16,20,23]. Another hypothesis is that the noxious effect on the brain is due to systemic induction of inflammatory cytokines [interleukin (IL)-1, IL-6 and tumor necrosis factor-α] by HCV which, *via* the blood-brain barrier, cause microglial activation and in-brain cytokine production[24]. This is the mechanism of the "sickness behaviour" syndrome, which includes non-specific depressive symptoms: fatigue, anhedonia, apathy, emotional lability, irritability, agitation, anorexia, psychomotor retardation, sleep disturbance, social withdrawal, hyperalgesia, decreased libido, and cognitive impairment[25-27].

HCV INFECTION AND IFN TREATMENT

Another approach to solve the problem of the existence, if any, of a direct effect of HCV infection on mood and HRQoL, is to examine the results of trials on HCV treatment. Such studies concern patients with chronic viral hepatitis; therefore, the dimension of liver disease cannot be dissociated from that of HCV infection *per se*. At any rate, large studies based on the SF36 health questionnaire have shown a significant improvement in quality of life scores in those patients with chronic hepatitis who obtain a sustained viral response[28,29]. However, these kinds of studies cannot remove the bias caused by patient satisfaction due to the awareness of being healed of hepatitis C.

In addition, the interpretation of the change of HRQoL in HCV-infected patients who are treated with IFN is biased by the fact that IFN itself is a proinflammatory cytokine that induces neuropsychiatric symptoms. In fact, 12%-41% of subjects with HCV hepatitis who are treated with IFN develop neuropsychiatric symptoms, even if they did not have a history of previous mental disorder[10]. An even higher percentage of individuals, ranging from 17%-58%, develop neuropsychiatric symptoms, mainly depression, if they had had a history of previous mental disorder[10]. The depressive syndrome induced by IFN has two expressions: (1) a depression-specific syndrome characterized by depressed mood, anhedonia, anxiety, subjective cognitive disturbance and sometimes suicidal ideation; and (2) a neurovegetative syndrome, characterized by fatigue, insomnia, anorexia, pain, psychomotor slowness. Generally, symptoms remit within 2-3 wk of discontinuation of IFN treatment[30]. Nevertheless, some patients may continue to complain of cognitive difficulties 12-24 mo after discontinuation of IFN treatment[31]. Obviously, these neuropsychiatric IFN side effects influence HRQoL, which depends on subjective perception and self-report of personal well-being. Therefore, the cessation of IFN treatment and, thus, of the discomfort caused by treatment itself, might explain a rebound perception of increased HRQoL.

LIVER TRANSPLANTATION

Liver transplantation (LT) is a life-saving intervention for many patients with end-stage liver disease: survival after this intervention ranges between 90%-70% at one and five years, respectively. Many studies have reported significant improvements in HRQoL and satisfactory psychological outcome after LT[32,33]. The topic was recently reviewed by Tome *et al*[34] who observed that general HRQoL improves dramatically after LT; however, when compared with the general population, the vast majority of LT patients have significant deficits in most HRQoL domains. In addition, while some domains, such as physical functioning and psychosocial adaptation, significantly improve after transplantation, psychological health shows a lower improvement than physical functioning. On the whole, sexual life does not improve significantly; however, results in the literature so far are extremely heterogeneous, suggesting that this effect is essentially unpredictable in individual patients. A relationship between the aetiology of liver disease and HRQoL has been reported: HCV infections impinge on HRQoL in the patients with recur-

rence of liver disease[35]. The impairment is mainly related to physical functioning and fatigue[35,36]. An intriguing issue is the interplay amongst HRQoL, psychological status and brain function. Interestingly, elevated levels of anxiety and neuroticism on pre-transplantation assessment were associated with poor psychological outcome one year after transplantation. These findings suggest that personality and affective status may be important determinants of long-term psychological health after LT. Accordingly, patients with HCV who experienced virologic recurrence within 6 mo of LT showed significantly greater depression, anxiety, phobic anxiety, and paranoid ideation than anti-HCV-negative patients[33].

In conclusion, independent of the underlying pathophysiologic mechanisms, HCV infection is a signal of vulnerability for poor HRQoL and should be considered in patient assessment.

REFERENCES

1 **Shepard CW,** Finelli L, Alter MJ. Global epidemilogy of hepatitic C virus infection. *Lancet Infect Dis* 2005; **5**: 558-567

2 **Armstrong GL**, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006; **144**: 705-714

3 **Poynard T**, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825-832

4 **Sangiovanni A**, Del Ninno E, Fasani P, De Fazio C, Ronchi G, Romeo R, Morabito A, De Franchis R, Colombo M. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. *Gastroenterology* 2004; **126**: 1005-1014

5 **Faustini A**, Colais P, Fabrizi E, Bargagli AM, Davoli M, Di Lallo D, Di Napoli A, Pezzotti P, Sorge C, Grillo R, Maresca C, Recchia O, Perucci CA. Hepatic and extra-hepatic sequelae, and prevalence of viral hepatitis C infection estimated from routine data in at-risk groups. *BMC Infect Dis* 2010; **10**: 97

6 **D'Amico G**, Morabito A, Pagliaro L, Marubini E. Survival and prognostic indicators in compensated and decompensated cirrhosis. *Dig Dis Sci* 1986; **31**: 468-475

7 **Ginés P**, Quintero E, Arroyo V, Terés J, Bruguera M, Rimola A, Caballería J, Rodés J, Rozman C. Compensated cirrhosis: natural history and prognostic factors. *Hepatology* 1987; **7**: 122-128

8 **Marchesini G**, Bianchi G, Amodio P, Salerno F, Merli M, Panella C, Loguercio C, Apolone G, Niero M, Abbiati R. Factors associated with poor health-related quality of life of patients with cirrhosis. *Gastroenterology* 2001; **120**: 170-178

9 **Younossi Z**, Kallman J, Kincaid J. The effects of HCV infection and management on health-related quality of life. *Hepatology* 2007; **45**: 806-816

10 **Quelhas R**, Lopes A. Psychiatric problems in patients infected with hepatitis C before and during antiviral treatment with interferon-alpha: a review. *J Psychiatr Pract* 2009; **15**: 262-281

11 **Constant A**, Castera L, Quintard B, Bernard PH, de Ledinghen V, Couzigou P, Bruchon-Schweitzer M. Psychosocial factors associated with perceived disease severity in patients with chronic hepatitis C: relationship with information sources and attentional coping styles. *Psychosomatics* 2005; **46**: 25-33

12 **Johnson ME**, Fisher DG, Fenaughty A, Theno SA. Hepatitis C virus and depression in drug users. *Am J Gastroenterol* 1998; **93**: 785-789

13 **Foster GR**, Goldin RD, Thomas HC. Chronic hepatitis C virus infection causes a significant reduction in quality of life in the absence of cirrhosis. *Hepatology* 1998; **27**: 209-212

14 **Rodger AJ**, Jolley D, Thompson SC, Lanigan A, Crofts N. The impact of diagnosis of hepatitis C virus on quality of life. *Hepatology* 1999; **30**: 1299-1301

15 **Weissenborn K**, Ennen JC, Bokemeyer M, Ahl B, Wurster U, Tillmann H, Trebst C, Hecker H, Berding G. Monoaminergic neurotransmission is altered in hepatitis C virus infected patients with chronic fatigue and cognitive impairment. *Gut* 2006; **55**: 1624-1630

16 **Forton DM**, Allsop JM, Main J, Foster GR, Thomas HC, Taylor-Robinson SD. Evidence for a cerebral effect of the hepatitis C virus. *Lancet* 2001; **358**: 38-39

17 **Forton DM**, Hamilton G, Allsop JM, Grover VP, Wesnes K, O'Sullivan C, Thomas HC, Taylor-Robinson SD. Cerebral immune activation in chronic hepatitis C infection: a magnetic resonance spectroscopy study. *J Hepatol* 2008; **49**: 316-322

18 **Weissenborn K**, Krause J, Bokemeyer M, Hecker H, Schüler A, Ennen JC, Ahl B, Manns MP, Böker KW. Hepatitis C virus infection affects the brain-evidence from psychometric studies and magnetic resonance spectroscopy. *J Hepatol* 2004; **41**: 845-851

19 **Forton DM**, Karayiannis P, Mahmud N, Taylor-Robinson SD, Thomas HC. Identification of unique hepatitis C virus quasispecies in the central nervous system and comparative analysis of internal translational efficiency of brain, liver, and serum variants. *J Virol* 2004; **78**: 5170-5183

20 **Weissenborn K**, Tryc AB, Heeren M, Worthmann H, Pflugrad H, Berding G, Bokemeyer M, Tillmann HL, Goldbecker A. Hepatitis C virus infection and the brain. *Metab Brain Dis* 2009; **24**: 197-210

21 **Laskus T**, Radkowski M, Bednarska A, Wilkinson J, Adair D, Nowicki M, Nikolopoulou GB, Vargas H, Rakela J. Detection and analysis of hepatitis C virus sequences in cerebrospinal fluid. *J Virol* 2002; **76**: 10064-10068

22 **Vargas HE**, Laskus T, Radkowski M, Wilkinson J, Balan V, Douglas DD, Harrison ME, Mulligan DC, Olden K, Adair D, Rakela J. Detection of hepatitis C virus sequences in brain tissue obtained in recurrent hepatitis C after liver transplantation. *Liver Transpl* 2002; **8**: 1014-1019

23 **Forton DM**, Thomas HC, Murphy CA, Allsop JM, Foster GR, Main J, Wesnes KA, Taylor-Robinson SD. Hepatitis C and cognitive impairment in a cohort of patients with mild liver disease. *Hepatology* 2002; **35**: 433-439

24 **Raison CL**, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. *Trends Immunol* 2006; **27**: 24-31

25 **Miller AH**, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. *Biol Psychiatry* 2009; **65**: 732-741

26 **Raison C**. The effects of hepatitis C and its treatment on mental health. *Focus* 2006; **21**: 4-6

27 **Raison CL**, Miller AH. The neuroimmunology of stress and depression. *Semin Clin Neuropsychiatry* 2001; **6**: 277-294

28 **Bernstein D**, Kleinman L, Barker CM, Revicki DA, Green J. Relationship of health-related quality of life to treatment adherence and sustained response in chronic hepatitis C patients. *Hepatology* 2002; **35**: 704-708

29 **McHutchison JG**, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; **123**: 1061-1069

30 **Valentine AD**, Meyers CA, Talpaz M. Treatment of neurotoxic side effects of interferon-alpha with naltrexone. *Cancer Invest* 1995; **13**: 561-566

31 **Lieb K**, Engelbrecht MA, Gut O, Fiebich BL, Bauer J, Janssen G, Schaefer M. Cognitive impairment in patients with chronic hepatitis treated with interferon alpha (IFNalpha): results from a prospective study. *Eur Psychiatry* 2006; **21**: 204-210
32 **Bryan S**, Ratcliffe J, Neuberger JM, Burroughs AK, Gunson BK, Buxton MJ. Health-related quality of life following liver transplantation. *Qual Life Res* 1998; **7**: 115-120
33 **O'Carroll RE**, Couston M, Cossar J, Masterton G, Hayes PC. Psychological outcome and quality of life following liver transplantation: a prospective, national, single-center study. *Liver Transpl* 2003; **9**: 712-720
34 **Tome S**, Wells JT, Said A, Lucey MR. Quality of life after liver transplantation. A systematic review. *J Hepatol* 2008; **48**: 567-577
35 **Bona MD**, Rupolo G, Ponton P, Iemmolo RM, Boccagni P, Destro C, Ermani M, Naccarato R, Burra P. The effect of recurrence of HCV infection of life after liver transplantation. *Transpl Int* 1998; **11** Suppl 1: S475-S479
36 **Singh N**, Gayowski T, Wagener MM, Marino IR. Quality of life, functional status, and depression in male liver transplant recipients with recurrent viral hepatitis C. *Transplantation* 1999; **67**: 69-72

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EDITORIAL

Animal models of nonalcoholic fatty liver disease/ nonalcoholic steatohepatitis

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is a condition in which excess fat accumulates in the liver of a patient without a history of alcohol abuse. Nonalcoholic steatohepatitis (NASH), a severe form of NAFLD, can progress to liver cirrhosis and hepatocellular carcinoma. NAFLD is regarded as a hepatic manifestation of metabolic syndrome and incidence has been increasing worldwide in line with the increased prevalence of obesity, type 2 diabetes, and hyperlipemia. Animal models of NAFLD/NASH give crucial information, not only in elucidating pathogenesis of NAFLD/NASH but also in examining therapeutic effects of various agents. An ideal model of NAFLD/NASH should correctly reflect both hepatic histopathology and pathophysiology of human NAFLD/NASH. Animal models of NAFLD/NASH are divided into genetic, dietary, and combination models. In this paper, we review commonly used animal models of NAFLD/NASH referring to their advantages and disadvantages.

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Key words: Animal model; Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Metabolic syndrome; Histopathology

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a condition in which excess fat accumulates in the liver of a patient without a history of alcohol abuse[1]. NAFLD is classified into simple steatosis and nonalcoholic steatohepatitis (NASH). In NASH, not only steatosis but also intralobular inflammation and hepatocellular ballooning are present, often accompanied by progressive fibrosis[2]. Long-standing NASH may progress to liver cirrhosis, and hepatocellular carcinoma (HCC) may be an outcome[3-5].

NAFLD is regarded as a hepatic manifestation of metabolic syndrome[6,7]. NAFLD has been increasing worldwide over recent decades in line with the increased prevalence of obesity, type 2 diabetes, and hyperlipemia. NAFLD/NASH is currently regarded as the most common chronic liver disease worldwide. It is estimated that about 20% of all adults have NAFLD and 2%-3% of adults have NASH[8].

The pathogenesis of NASH has not been completely elucidated, and treatments for NASH other than lifestyle modification by diet and exercise have not been fully established[5]. Studies of NAFLD/NASH using human materials have limitations, because the occurrence and progression of NAFLD/NASH require a long period of several decades, and ethical limitations exist in admin-

istering drugs to patients or collecting liver tissues from patients. Animal models of NAFLD/NASH give crucial information, not only in elucidating the pathogenesis of NAFLD/NASH but also in examining therapeutic effects of various agents. These animal models need to reflect correctly both the histopathology and pathophysiology of human NAFLD/NASH. Recently, several review articles on animal models of NAFLD/NASH have been published[9,10]. In this paper, we firstly review the histopathology and pathogenesis of NAFLD/NASH, and thereafter, we review current animal models of NAFLD/NASH referring to their advantages and disadvantages, with emphasis on a fructose-enriched diet model that was established by us.

HISTOPATHOLOGY OF NAFLD

The most important histological characteristic of NAFLD is steatosis of hepatocytes. Steatosis of hepatocytes is classified into macrovesicular and microvesicular. Macrovesicular steatosis is characterized by large vacuoles occupying almost the entire cytoplasm and pushing the nucleus to the periphery of the cell. Microvesicular steatosis is characterized by multiple small lipid vacuoles, and the nucleus is located at the center of the cell. Typically, steatosis in NAFLD is centrilobular and macrovesicular. However, steatosis may be present throughout the lobule, and microvesicular steatosis may also be present. Steatosis in more than 5% of hepatocytes is necessary for a diagnosis of NAFLD[11].

In NASH, not only steatosis but also intralobular inflammation and hepatocellular ballooning are present, and this is usually accompanied by fibrosis. Mallory's hyaline (MH) may also be present. Intralobular inflammation in NASH is typically mild and of a mixed type and includes a small number of lymphocytes, macrophages, and neutrophils. Neutrophils tend to infiltrate in the area of marked steatosis and around MH. Portal inflammation is usually mild; however, relatively intense chronic inflammation may be present in the portal area.

Hepatocellular ballooning is a form of liver cell injury recognized as a swollen hepatocyte with a rarefied cytoplasm. It is most apparent near steatotic liver cells, typically in zone 3.

MH is an eosinophilic and amorphous structure in the cytoplasm of hepatocytes and is observed in alcoholic hepatitis, NASH, chronic cholestasis, and HCC[2]. MH in NASH is most frequently found in the areas of perisinusoidal fibrosis in zone 3 and may be accompanied by neutrophilic infiltration. MH in NASH is usually less distinct than that in alcoholic hepatitis. Immunohistochemically, MH is positive for cytokeratin (CK) 8, CK 18, p62, and ubiquitin[2].

Other pathological features that are often observed in NASH include fat cysts and lipogranulomas, and glycogenated nuclei frequently occur in hepatocytes in zone 1. Furthermore, megamitochondria and iron deposition may be observed in NASH.

Fibrosis usually originates in the perisinusoidal regions of zone 3 (perisinusoidal fibrosis) and may also be present in the periportal area. As the disease progresses, bridging fibrosis and liver cirrhosis may develop. In the process, steatosis and lesion activity may resolve, resulting in a diagnosis of "cryptogenic cirrhosis" (burn-out NASH).

Brunt *et al*[12] proposed the grading and staging system of NASH, and the NASH Clinical Research Network designed and validated the NAFLD activity score[13]. These systems are frequently used in both clinical studies and animal experiments. Intralobular changes are milder and portal inflammation is more severe in pediatric NAFLD than in adult NAFLD[14,15]. Furthermore, steatosis in pediatric NAFLD is not necessarily predominant in zone 3[15,16].

PATHOGENESIS OF NAFLD/NASH

The "two-hit" hypothesis proposed by Day *et al*[17] is widely accepted as the pathogenesis of NAFLD/NASH; the first hit causes fat accumulation in hepatocytes, and the second hit causes inflammation and fibrosis. Fat accumulation in the liver is closely associated with metabolic derangements that are related to central obesity and insulin resistance[18]. The most reproducible risk factors for NAFLD/NASH are central obesity, insulin resistance, fasting hyperglycemia, and hypertriglyceridemia[19], and NAFLD/NASH is regarded as a hepatic manifestation of metabolic syndrome[6,7].

Hepatic steatosis occurs when the rate of import or synthesis of fatty acids by hepatocytes exceeds the rate of export or catabolism[20,21]. Accordingly, the following 4 mechanisms are possible causes of lipid accumulation within the liver: (1) increased delivery and uptake into hepatocytes of long-chain fatty acids (LCFA) due to excess dietary intake or release from adipose tissue; (2) increased *de novo* hepatic LCFA and triglyceride synthesis; (3) failure of very low-density lipoprotein (VLDL) synthesis and triglyceride export; and (4) failure of LCFA elimination due to impaired hepatic mitochondrial β-oxidation[22].

Once steatosis has developed, the liver is sensitized; thus, an inflammatory response may be precipitated by a variety of stimuli[22]. Oxidative stress, pro-inflammatory cytokine [e.g., tumor necrosis factor (TNF)-α]-mediated hepatocyte injury, altered lipid partitioning and hepatotoxicity mediated by free fatty acids, abnormal intrahepatic cholesterol loading, hyperinsulinemia, hyperleptinemia, hypoadiponectinemia, and apoptosis are all thought to be important second hits causing NASH[22-24].

ANIMAL MODELS OF NAFLD/NASH

An ideal animal model of NAFLD/NASH should reflect hepatic histopathology and pathophysiology of human NAFLD/NASH. Accordingly, the liver of the animal model of NASH should show steatosis, intralobular inflammation, hepatocellular ballooning, and, ideally, perisinusoidal fibrosis in zone 3 and susceptibility to liver

Table 1 Biochemical and pathological characteristics of animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis

Model	Obesity	Insulin resistance	Steatosis	Steatohepatitis	Fibrosis
SREBP-1c transgenic mice	No (decreased adiposity)	Yes	Yes	Yes	Yes
Ob/ob mice	Yes	Yes	Yes	No (does not develop spontaneously)	No (resistant to fibrosis)
Db/db mice	Yes	Yes	Yes	No (does not develop spontaneously)	No (does not develop spontaneously)
KK-A^y mice	Yes	Yes	Yes	No (does not develop spontaneously)	No (does not develop spontaneously)
PTEN null mice	No	No	Yes	Yes	Yes
PPAR-α knockout mice	No	No	No (steatosis occurs in the starved state)	No	No
AOX null mice	No	No	Yes	Yes	No
MAT1A null mice	No	No	Yes	Yes	Yes
Methionine and choline deficiency	No (decreased weight and adiposity)	Hepatic insulin resistance	Yes	Yes (severe)	Yes
High fat	Yes	Yes	Yes	Yes (mild)	Yes
Cholesterol and cholate (atherogenic diet)	No (decreased weight)	Hepatic insulin resistance	Yes	Yes	Yes
Fructose	No	Yes	Yes	No/Yes	No

SREBP: Sterol regulatory element binding protein; PTEN: Phosphatase and tensin homologue deleted on chromosome 10; AOX: Acyl-coenzyme A oxidase; MAT1A: Methionine adenosyltransferase-1A; PPAR: Peroxisome proliferator-activated receptor.

tumors. Furthermore, the animal should show metabolic abnormalities such as obesity, insulin resistance, fasting hyperglycemia, dyslipidemia, and altered adipokine profile. It is questionable whether the results of a study using an animal model that does not completely fulfill these conditions can be extrapolated to human disease. Animal models of NAFLD/NASH are classified into genetic models, nutritional models, and combination models of genetic and nutritional factors. Numerous animal models of NAFLD/NASH have been reported to date; however, no animal model completely reflects hepatic histopathology and pathophysiology of human NAFLD/NASH. It is, therefore, important to select the animal model that best conforms to the aim of the study. Biochemical and pathological characteristics of animal models described in this paper are summarized in Table 1. As described below, in many of the genetic models of NASH [e.g., sterol regulatory element binding protein (SREBP)-1c transgenic mice and phosphatase and tensin homologue deleted on chromosome 10 (PTEN) null mice], hepatic steatosis occurs first, and steatohepatitis develops later. Ob/ob, db/db, and KK-A^y mice do not progress to steatohepatitis spontaneously. On the other hand, in a dietary model induced by methionine and choline deficiency, steatohepatitis occurs very quickly.

GENETIC MODELS

SREBP-1c transgenic mice

In the fat tissue of these mice, SREBP-1c, a lipogenic transcription factor, is overexpressed. This creates a model of congenital lipodystrophy in which severe insulin resistance and diabetes develop secondary to impaired adipose differentiation[25]. In these mice, decreased fat tissue with lipid accumulation in the liver is observed, and marked hepatic steatosis occurs by 8 d of age. When fed a standard diet, steatosis, lobular inflammation, and perivenular and pericellular fibrosis develop at 20 wk[26]; ballooned hepatocytes and MH are also observed. These mice appear to be appropriate for the lipodystrophy-associated steatohepatitis model. However, it is questionable whether they can be used as a model for typical NAFLD/NASH, because visceral fat characteristically increases in human NAFLD/NASH.

Ob/ob mice

Ob/ob mice possess a spontaneous mutation in the leptin gene (leptin-deficient). Leptin is an adipokine produced by white adipose tissue and operates on the hypothalamic ventral median nucleus exerting a marked anorexic effect[22]. Ob/ob mice are hyperphagic, inactive, and extremely obese, and show hyperglycemia, insulin resistance, and hyperinsulinemia[27]. Ob/ob mice develop spontaneous hepatic steatosis[28]; this does not, however, progress to steatohepatitis spontaneously. Secondary insults such as a methionine- and choline-deficient (MCD) diet, a high fat (HF) diet, or low-dose lipopolysaccharide (endotoxin) are needed to trigger steatohepatitis in ob/ob mice[29,30]. Another feature of ob/ob mice is that they are protected against fibrosis[31], a phenomenon which led to the characterization of leptin as an essential mediator of hepatic fibrogenesis[31,32]. Mutations in the *ob* gene are not prevalent in obese subjects or NASH patients, and leptin levels correlate poorly with the development of NASH[33].

Db/db mice

Db/db mice possess a natural mutation in the leptin receptor (*Ob-Rb*) gene[34] and, therefore, show normal or elevated levels of leptin but are resistant to the effects of leptin. These mice are obese, insulin resistant, and dia-

betic, and develop macrovesicular hepatic steatosis. They develop NASH when a second hit such as an MCD diet is added[35]. When db/db mice are fed an MCD diet, significant liver fibrosis is observed as compared to that in ob/ob mice[36]. The advantage of ob/ob and db/db mice is that the phenotype of these mice simulates the human condition of metabolic syndrome in many aspects. However, these mice have the disadvantage that they do not spontaneously develop steatohepatitis or liver fibrosis.

KK-A^y mice

A heterozygous mutation of the agouti gene (*KK-Ay/a*) results in a loss of melanocortin and an obese phenotype due to hyperphagia from impaired hypothalamic appetite suppression[9]. Although these mice develop hepatic steatosis in conjunction with obesity and insulin resistance, significant steatohepatitis does not occur spontaneously. It was reported that KK-A^y mice exhibited increased susceptibility to MCD diet-induced steatohepatitis, where hypoadiponectinemia most likely played a key role in exacerbation of both inflammatory and profibrogenic responses[37].

PTEN 10 null mice

PTEN is a tumor suppressor gene encoding a lipid phosphatase whose major substrate is phosphatidylinositol-3,4,5-triphosphate (PIP3). PTEN is a negative regulator of several signaling pathways such as phosphatidylinositol 3-kinase and serine-threonine protein kinase B (PKB, or Akt)[38]. These pathways regulate apoptosis, cell proliferation, and tumor formation[38]. Liver-specific *Pten* knockout mice (*AlbCrePTEN flox/flox* mice) show extensive hepatomegaly and steatohepatitis, and the histopathology is similar to that in human NASH[39]. Steatosis is observed at 10 wk of age, and steatohepatitis with fibrosis is observed at 40 wk. Hepatocellular adenomas occur with an incidence of 47% by 44 wk, and by 74-78 wk, all of the livers show adenomas, whereas 66% develop HCCs[40]. *Pten* knockout mice are hypersensitive to insulin, and *Pten* null hepatocytes have high proliferative activity *in vitro*[40]. The advantage of this model is that the histological phenotype resembles that of human NASH. The disadvantage of this model is that it is hypersensitive to insulin.

Peroxisome proliferator-activated receptor-α knockout mice

Peroxisome proliferator-activated receptor-α (PPAR-α) is a key regulator of genes involved in peroxisomal, mitochondrial, and microsomal fatty acid oxidation systems in the liver, and a significant decrease in PPAR-α is observed in the HF diet model[41]. Fat does not accumulate in the liver of mice with a homozygous mutation of the *PPAR*-α gene under conditions of normal feeding, but in the starved state, hepatic steatosis occurs because fatty acid oxidation is inhibited[42].

Acyl-coenzyme A oxidase null mice

Acyl-coenzyme A oxidase (AOX) is the rate-limiting enzyme of peroxisomal β-oxidation of LCFA. *AOX* null (*AOX* -/-) mice have defective peroxisomal β-oxidation of LCFA, which accumulate in the liver and lead to steatohepatitis. *AOX* -/- mice begin to exhibit microvesicular fatty change of hepatocytes in zones 2 and 3 of liver lobules at 7 d of age[43]. By 30 d of age, the *AOX* -/- mouse liver shows increased severity of steatosis of liver parenchymal cells and concomitant occurrence of focal inflammatory cell infiltrate[43]. At 2 mo of age, clusters of hepatocytes with peroxisome-rich eosinophilic granular cytoplasm are observed in periportal areas[43]. By 4-5 mo of age, increased expressions of PPAR-α, cytochrome P450 (Cyp) 4a10 and Cyp 4a14, and increased levels of H_2O_2 are observed[44]. However, compensative increase of fatty acid oxidation is observed by 6-7 mo of age, and hepatic steatosis recovers by regeneration of hepatocytes. *AOX* -/- mice develop hepatocellular adenomas and HCCs by 15 mo of age[44].

Methionine adenosyltransferase-1A null mice

Methionine adenosyltransferase-1A (MAT1A) is a liver-specific rate-limiting enzyme of methionine metabolism and catalyzes the formation of S-adenosylmethionine. *MAT1A* null mice have decreased levels of antioxidants, including glutathione, and decreased expressions of genes involved in lipid oxidation such as Cyp 4a10 and Cyp 4a14[44]. *MAT1A* null mice spontaneously develop steatohepatitis after 8 mo of age, and proliferation of hepatocytes increases, resulting in tumor development[45,46]. The mice are susceptible to choline-deficient diet-induced fatty liver at 3 mo of age[45]. Although *MAT1A* null mice are hyperglycemic, their insulin levels are normal and they do not appear to develop other features of metabolic syndrome[46].

DIETARY MODELS OF NASH

Methionine and choline deficiency

The MCD diet contains high sucrose and fat (40% sucrose, 10% fat) but lacks methionine and choline, which are essential for hepatic β-oxidation and production of VLDL[22]. In addition, choline deficiency impairs hepatic VLDL secretion[47]. As a result, lipid is deposited in the liver. Furthermore, oxidative stress[48,49] and changes in cytokines and adipocytokines[50] occur, contributing to the liver injury. Antagonizing oxidative stress by increasing antioxidant capacities attenuates the degree of steatohepatitis and stresses the importance of reactive oxygen species in this model[51].

Serum alanine aminotransferase (ALT) level is consistently increased after MCD-diet feeding in mice[52]. Steatohepatitis occurs at day 10[52], and perisinusoidal fibrosis is observed by 8-10 wk in mice[48,53]. After 10 wk of MCD feeding, extensive macrovesicular steatosis is observed in

all areas except for the periportal region, and many necro-inflammatory foci containing lymphocytes and neutrophils are observed in mice[54]. Although the MCD model causes more severe inflammation, oxidative stress, mitochondrial damage, apoptosis, and fibrogenesis than other animal nutritional models of NASH[55], the severity of NASH in rodents fed the MCD diet may depend on the species, gender, and strain of the animal[56]. Kirsch *et al*[57] compared the effects of MCD diet using male and female Wistar, Long-Evans, and Sprague-Dawley rats, and C57BL/6 mice. As a result, the Wistar strain and the male sex were associated with the greatest degree of steatosis in rats. Of the groups studied, male C57BL/6 mice developed the most inflammation and necrosis, and best approximated the histological features of NASH.

The main disadvantage of the MCD model is that the metabolic profile of the model is opposite to that of typical human NASH. Namely, mice fed the MCD diet show significant weight loss (often, more than 20% weight loss after 3 wk), low fasting blood sugar, peripheral insulin sensitivity, low serum insulin and leptin levels, and unchanged or increased serum adiponectin levels[50,58-61]. To improve these problems, genetically obese mice, such as ob/ob and db/db mice, are occasionally used as the MCD-fed animal. The main advantages of the MCD diet are that it is easy to obtain and use.

HF diet

Lieber *et al*[62] reported a diet model of NASH by using an HF diet (71% of energy from fat, 11% from carbohydrates, and 18% from proteins). Rats fed this diet *ad libitum* for 3 wk showed elevated plasma insulin levels reflecting insulin resistance. Rats fed the HF diet developed marked panlobular steatosis, and the hepatic lipid concentrations of these rats were approximately twice those of control rats fed the standard Lieber-DeCarli diet (35% fat, 47% carbohydrates, and 18% protein). Like human NASH, the rats fed the HF diet developed oxidative damage in the liver. When dietary consumption was restricted, steatosis and inflammation in the liver, oxidative stress, and plasma insulin levels were decreased. Feeding of an HF emulsion to Sprague-Dawley rats also induced changes closely resembling human NASH[63]. In the longitudinal study by Ito *et al*[64], chronic administration of an HF diet (60% of calories from fat) caused steatohepatitis in male C57BL/6J mice.

Intragastric overfeeding of mice with an HF diet up to 85% in excess of standard intake for 9 wk has been reported to replicate the histopathological and pathogenic features of NASH[65]. Overfed C57BL/6 mice became obese (71% higher body weight), had increased visceral fat (white adipose tissue: WAT), and showed hyperglycemia, hyperinsulinemia, hyperleptinemia, glucose intolerance, and insulin resistance. Almost half (46%) of the animals developed NASH, and their plasma ALT levels showed 9- to 10-fold increases. Neutrophilic infiltration and peri-sinusoidal fibrosis reminiscent of human NASH were observed. The WAT exhibited increased TNF-α and leptin expressions and reduced adiponectin expression.

An HF diet is widely used to cause hepatic steatosis and NASH in experimental animals. However, it seems that the HF diet model produces variable results with regard to the degree of steatosis, inflammation, and fibrosis, and the results depend on rodent species and strain, the fat content in the diet, the composition of dietary fat, and the duration of treatment. For example, Sprague-Dawley rats appear susceptible to steatohepatitis development when fed an HF diet, and this is likely associated with their susceptibility to diet-induced obesity[54]. On the other hand, it was reported that long term high saturated fat feeding did not induce hepatic steatosis and NASH in Wistar rats[66]. In mice, it was reported that BALB/c male mice accumulated more hepatic lipid than C57BL/6J male mice when fed an HF diet[67]. In general, compared with the MCD model, the degree of liver injury in the HF model is less severe[10]. Among the HF models, the histopathology and pathophysiology of the intragastric overfeeding method most resemble those of human NASH. However, this method is difficult to implement, because it requires specific equipment and expertise. The optimization of the composition of the HF diet to reliably cause NASH in animals by *ad libitum* administration warrants future investigation. Recently, Ogasawara *et al*[68] reported a combined model of HF diet and gold thioglucose (GTG) administration. GTG is known to induce lesions in the ventromedial hypothalamus, leading to hyperphagia and obesity. They administered GTG intraperitoneally to C57BL/6 mice, and thereafter, fed an HF diet to the mice for 12 wk. As a result, obesity with increased abdominal adiposity, glucose intolerance, insulin resistance, and steatohepatitis with hepatocyte ballooning, MH, and pericellular fibrosis were induced.

Cholesterol and cholate

Matsuzawa *et al*[69] fed mice an atherogenic diet containing 1.25% cholesterol and 0.5% cholate and observed the progressive formation of steatosis, inflammation, and fibrosis in a time-dependent manner over 6-24 wk. In the model, hepatocellular ballooning, characteristic of human NASH, was observed at 24 wk. When 60% fat (cocoa butter) was added to the diet, development of these histopathological features was accelerated, and hepatocellular ballooning was observed at 12 wk. Furthermore, the atherogenic diet induced oxidative stress. Thus, it is conceivable that a combination diet of HF, cholesterol, and cholate in animals would cause histological features reminiscent of human NASH. However, the mice fed this diet were systematically insulin sensitive, albeit they showed hepatic insulin resistance. In fact, the mice lost 9% body weight during the experiment and had small epididymal fat pads and low plasma triglyceride levels compared with those in control mice. Therefore, this model appears to differ from human NASH in metabolic status.

Fructose

NAFLD/NASH is regarded as a hepatic manifestation of

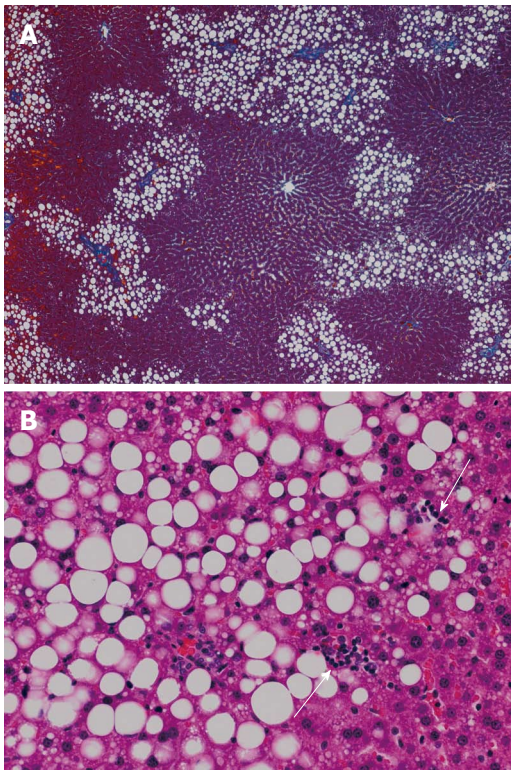

Figure 1 Liver histology of rats fed a high-fructose diet for 5 wk. A: Hepatic steatosis, mainly distributed in zone 1, is observed (azan stain, × 40); B: Both macrovesicular and microvesicular steatosis are evident as well as scattered necroinflammatory foci (arrows) (hematoxylin and eosin stain, × 200).

metabolic syndrome. Experimental animals fed a fructose-enriched diet are recognized as good models of metabolic syndrome. We examined livers of Wistar rats fed a high-fructose (70%) diet for 5 wk and found significantly higher macrovesicular steatosis (Figure 1A) and intralobular inflammation (Figure 1B) grades, liver:body weight ratios, and hepatic triglyceride concentrations than those in control rats[70]. In this study, the distribution of steatosis in the rats fed a high-fructose diet was characteristically predominant in zone 1. This pattern differs from that of human adult NAFLD, in which steatosis is usually predominant in zone 3. Rats fed a high-fructose diet showed significantly higher expressions of interleukin (IL)-6 protein and TNF-α protein in the liver compared with those in control rats (unpublished data). By adding plant leaf extract to the high-fructose diet, hepatic fatty change (Figure 2) and expression of IL-6 protein in the liver were completely suppressed (unpublished data).

Other groups also reported induction of NAFLD/NASH by fructose in experimental animals. Ackerman *et al*[71] found that male Sprague-Dawley rats fed a fructose-enriched (60%) diet developed macrovesicular and microvesicular steatosis in the liver. Armutcu *et al*[72] reported that male Wistar albino rats provided with drinking water containing 10% fructose for 10 d developed macrovesicular and microvesicular steatosis but did not develop inflammation in the liver. In mice, by adding 30% fructose to the drinking water, 3- to 4-fold increases in

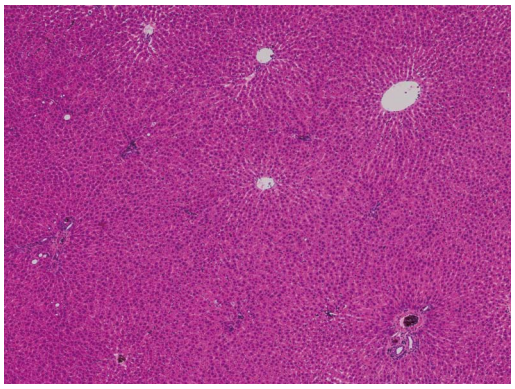

Figure 2 Liver histology of rats fed a high-fructose diet + plant leaf extract. Hepatic fatty change is completely suppressed by the plant leaf extract (hematoxylin and eosin stain, × 40).

hepatic triglyceride levels and marked increases in hepatic steatosis and weight were observed at 8 wk[73]. Recently, it was reported that C57BL/6 mice fed a high-fat high-carbohydrate diet and provided with drinking water containing 55% fructose for 16 wk developed a NASH-like phenotype with significant fibrosis as well as obesity[74]. Inflammation caused by endogenous toxins of fructose metabolites is suggested as one of the mechanisms of the second hit in the pathogenesis of NASH[75].

COMBINED MODEL OF GENETIC MODIFICATION AND NUTRITIONAL/DIETARY CHALLENGES

Many animal models combine naturally occurring genetic mutations or targeted gene modifications with dietary or chemical challenges so that the histopathology and pathophysiology of the models more closely resemble those of human NAFLD. Sahai *et al*[36] fed an MCD diet to ob/ob and db/db mice and observed that db/db mice had significantly higher serum ALT levels and more severe hepatic inflammation and fibrosis than those in ob/ob and wild-type mice. PPAR-α null mice fed an MCD diet show more severe steatohepatitis than that in wild-type mice fed the same diet[76,77]. Many other models combining genetic abnormalities with nutritional challenges have been reported[78-80].

CONCLUSION

As reviewed in this paper, many animal models of NASH have been developed to date. These animal models do not replicate the full spectrum of the disease in humans; however, they can be used in verifying hypotheses on the pathogenesis of NASH and in performing interventional studies. We hope that animal models which more closely reflect the histopathology and pathophysiology of human NASH will be developed in the future, and information on pathogenesis and treatment of NASH will increase by using these models.

REFERENCES

1 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231
2 **Takahashi Y,** Fukusato T. Pathology of nonalcoholic steatohepatitis. In: Current Research in Hepatology 2. Trivandrum: Research Media, 2008: 99-112
3 **Powell EE**, Cooksley WG, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology* 1990; **11**: 74-80
4 **Harrison SA**, Torgerson S, Hayashi PH. The natural history of nonalcoholic fatty liver disease: a clinical histopathological study. *Am J Gastroenterol* 2003; **98**: 2042-2047
5 **Cohen JC**, Horton JD, Hobbs HH. Human fatty liver disease: old questions and new insights. *Science* 2011; **332**: 1519-1523
6 **Marchesini G**, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923
7 **Machado M**, Cortez-Pinto H. Non-alcoholic steatohepatitis and metabolic syndrome. *Curr Opin Clin Nutr Metab Care* 2006; **9**: 637-642
8 **Neuschwander-Tetri BA**. Nonalcoholic steatohepatitis and the metabolic syndrome. *Am J Med Sci* 2005; **330**: 326-335
9 **Schattenberg JM**, Galle PR. Animal models of non-alcoholic steatohepatitis: of mice and man. *Dig Dis* 2010; **28**: 247-254
10 **Hebbard L**, George J. Animal models of nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 35-44
11 **Brunt EM,** Tiniakos DG. Alcoholic and non-alcoholic fatty liver disease. In: Odze RD, Goldblum JR, Crawford JM, editors. Pathology of the GI Tract, Liver, Biliary Tract and Pancreas. Philadelphia: Saunders, 2009: 1087-1114
12 **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
13 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321
14 **Schwimmer JB**, Behling C, Newbury R, Deutsch R, Nievergelt C, Schork NJ, Lavine JE. Histopathology of pediatric nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 641-649
15 **Takahashi Y**, Inui A, Fujisawa T, Takikawa H, Fukusato T. Histopathological characteristics of non-alcoholic fatty liver disease in children: Comparison with adult cases. *Hepatol Res* 2011; **41**: 1066-1074
16 **Carter-Kent C**, Brunt EM, Yerian LM, Alkhouri N, Angulo P, Kohli R, Ling SC, Xanthakos SA, Whitington PF, Charatcharoenwitthaya P, Yap J, Lopez R, McCullough AJ, Feldstein AE. Relations of steatosis type, grade, and zonality to histological features in pediatric nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2011; **52**: 190-197
17 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845
18 **Takahashi Y**, Fukusato T. Pediatric nonalcoholic fatty liver disease: overview with emphasis on histology. *World J Gastroenterol* 2010; **16**: 5280-5285
19 **Farrell GC**, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; **43**: S99-S112
20 **Koteish A**, Diehl AM. Animal models of steatosis. *Semin Liver Dis* 2001; **21**: 89-104
21 **Bradbury MW**, Berk PD. Lipid metabolism in hepatic steatosis. *Clin Liver Dis* 2004; **8**: 639-671
22 **Anstee QM**, Goldin RD. Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. *Int J Exp Pathol* 2006; **87**: 1-16
23 **Day CP**, Saksena S. Non-alcoholic steatohepatitis: definitions and pathogenesis. *J Gastroenterol Hepatol* 2002; **17** Suppl 3: S377-S384
24 **Marra F**, Gastaldelli A, Svegliati Baroni G, Tell G, Tiribelli C. Molecular basis and mechanisms of progression of non-alcoholic steatohepatitis. *Trends Mol Med* 2008; **14**: 72-81
25 **Shimomura I**, Hammer RE, Richardson JA, Ikemoto S, Bashmakov Y, Goldstein JL, Brown MS. Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. *Genes Dev* 1998; **12**: 3182-3194
26 **Nakayama H**, Otabe S, Ueno T, Hirota N, Yuan X, Fukutani T, Hashinaga T, Wada N, Yamada K. Transgenic mice expressing nuclear sterol regulatory element-binding protein 1c in adipose tissue exhibit liver histology similar to nonalcoholic steatohepatitis. *Metabolism* 2007; **56**: 470-475
27 **Bray GA**, York DA. Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiol Rev* 1979; **59**: 719-809
28 **Diehl AM**. Lessons from animal models of NASH. *Hepatol Res* 2005; **33**: 138-144
29 **Brix AE**, Elgavish A, Nagy TR, Gower BA, Rhead WJ, Wood PA. Evaluation of liver fatty acid oxidation in the leptin-deficient obese mouse. *Mol Genet Metab* 2002; **75**: 219-226
30 **Yang SQ**, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci USA* 1997; **94**: 2557-2562
31 **Leclercq IA**, Farrell GC, Schriemer R, Robertson GR. Leptin is essential for the hepatic fibrogenic response to chronic liver injury. *J Hepatol* 2002; **37**: 206-213
32 **Ikejima K**, Honda H, Yoshikawa M, Hirose M, Kitamura T, Takei Y, Sato N. Leptin augments inflammatory and profibrogenic responses in the murine liver induced by hepatotoxic chemicals. *Hepatology* 2001; **34**: 288-297
33 **Chalasani N**, Crabb DW, Cummings OW, Kwo PY, Asghar A, Pandya PK, Considine RV. Does leptin play a role in the pathogenesis of human nonalcoholic steatohepatitis? *Am J Gastroenterol* 2003; **98**: 2771-2776
34 **Chen H**, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI, Morgenstern JP. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* 1996; **84**: 491-495
35 **Wortham M**, He L, Gyamfi M, Copple BL, Wan YJ. The transition from fatty liver to NASH associates with SAMe depletion in db/db mice fed a methionine choline-deficient diet. *Dig Dis Sci* 2008; **53**: 2761-2774
36 **Sahai A**, Malladi P, Pan X, Paul R, Melin-Aldana H, Green RM, Whitington PF. Obese and diabetic db/db mice develop marked liver fibrosis in a model of nonalcoholic steatohepatitis: role of short-form leptin receptors and osteopontin. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G1035-G1043
37 **Okumura K**, Ikejima K, Kon K, Abe W, Yamashina S, Enomoto N, Takei Y, Sato N. Exacerbation of dietary steatohepatitis and fibrosis in obese, diabetic KK-A(y) mice. *Hepatol Res* 2006; **36**: 217-228
38 **Stiles B**, Wang Y, Stahl A, Bassilian S, Lee WP, Kim YJ, Sherwin R, Devaskar S, Lesche R, Magnuson MA, Wu H. Liver-specific deletion of negative regulator Pten results in fatty liver and insulin hypersensitivity [corrected]. *Proc Natl Acad Sci USA* 2004; **101**: 2082-2087
39 **Sato W**, Horie Y, Kataoka E, Ohshima S, Dohmen T, Iizuka M, Sasaki J, Sasaki T, Hamada K, Kishimoto H, Suzuki A, Watanabe S. Hepatic gene expression in hepatocyte-specific Pten deficient mice showing steatohepatitis without ethanol challenge. *Hepatol Res* 2006; **34**: 256-265
40 **Horie Y**, Suzuki A, Kataoka E, Sasaki T, Hamada K, Sasaki J, Mizuno K, Hasegawa G, Kishimoto H, Iizuka M, Naito M, Enomoto K, Watanabe S, Mak TW, Nakano T. Hepatocyte-

specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. *J Clin Invest* 2004; **113**: 1774-1783
41 **Svegliati-Baroni G**, Candelaresi C, Saccomanno S, Ferretti G, Bachetti T, Marzioni M, De Minicis S, Nobili L, Salzano R, Omenetti A, Pacetti D, Sigmund S, Benedetti A, Casini A. A model of insulin resistance and nonalcoholic steatohepatitis in rats: role of peroxisome proliferator-activated receptor-alpha and n-3 polyunsaturated fatty acid treatment on liver injury. *Am J Pathol* 2006; **169**: 846-860
42 **Kersten S**, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *J Clin Invest* 1999; **103**: 1489-1498
43 **Cook WS**, Jain S, Jia Y, Cao WQ, Yeldandi AV, Reddy JK, Rao MS. Peroxisome proliferator-activated receptor alpha-responsive genes induced in the newborn but not prenatal liver of peroxisomal fatty acyl-CoA oxidase null mice. *Exp Cell Res* 2001; **268**: 70-76
44 **London RM**, George J. Pathogenesis of NASH: animal models. *Clin Liver Dis* 2007; **11**: 55-74, viii
45 **Lu SC**, Alvarez L, Huang ZZ, Chen L, An W, Corrales FJ, Avila MA, Kanel G, Mato JM. Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. *Proc Natl Acad Sci USA* 2001; **98**: 5560-5565
46 **Martínez-Chantar ML**, Corrales FJ, Martínez-Cruz LA, García-Trevijano ER, Huang ZZ, Chen L, Kanel G, Avila MA, Mato JM, Lu SC. Spontaneous oxidative stress and liver tumors in mice lacking methionine adenosyltransferase 1A. *FASEB J* 2002; **16**: 1292-1294
47 **Yao ZM**, Vance DE. Reduction in VLDL, but not HDL, in plasma of rats deficient in choline. *Biochem Cell Biol* 1990; **68**: 552-558
48 **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
49 **Chowdhry S**, Nazmy MH, Meakin PJ, Dinkova-Kostova AT, Walsh SV, Tsujita T, Dillon JF, Ashford ML, Hayes JD. Loss of Nrf2 markedly exacerbates nonalcoholic steatohepatitis. *Free Radic Biol Med* 2010; **48**: 357-371
50 **Larter CZ**, Yeh MM, Williams J, Bell-Anderson KS, Farrell GC. MCD-induced steatohepatitis is associated with hepatic adiponectin resistance and adipogenic transformation of hepatocytes. *J Hepatol* 2008; **49**: 407-416
51 **Oz HS**, Im HJ, Chen TS, de Villiers WJ, McClain CJ. Glutathione-enhancing agents protect against steatohepatitis in a dietary model. *J Biochem Mol Toxicol* 2006; **20**: 39-47
52 **Dela Peña A**, Leclercq I, Field J, George J, Jones B, Farrell G. NF-kappaB activation, rather than TNF, mediates hepatic inflammation in a murine dietary model of steatohepatitis. *Gastroenterology* 2005; **129**: 1663-1674
53 **Ip E**, Farrell G, Hall P, Robertson G, Leclercq I. Administration of the potent PPARalpha agonist, Wy-14,643, reverses nutritional fibrosis and steatohepatitis in mice. *Hepatology* 2004; **39**: 1286-1296
54 **Larter CZ**, Yeh MM. Animal models of NASH: getting both pathology and metabolic context right. *J Gastroenterol Hepatol* 2008; **23**: 1635-1648
55 **Gao D**, Wei C, Chen L, Huang J, Yang S, Diehl AM. Oxidative DNA damage and DNA repair enzyme expression are inversely related in murine models of fatty liver disease. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G1070-G1077
56 **Fan JG**, Qiao L. Commonly used animal models of nonalcoholic steatohepatitis. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 233-240
57 **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
58 **Rinella ME**, Green RM. The methionine-choline deficient dietary model of steatohepatitis does not exhibit insulin resistance. *J Hepatol* 2004; **40**: 47-51
59 **Schattenberg JM**, Singh R, Wang Y, Lefkowitch JH, Rigoli RM, Scherer PE, Czaja MJ. JNK1 but not JNK2 promotes the development of steatohepatitis in mice. *Hepatology* 2006; **43**: 163-172
60 **Nagasawa T**, Inada Y, Nakano S, Tamura T, Takahashi T, Maruyama K, Yamazaki Y, Kuroda J, Shibata N. Effects of bezafibrate, PPAR pan-agonist, and GW501516, PPARdelta agonist, on development of steatohepatitis in mice fed a methionine- and choline-deficient diet. *Eur J Pharmacol* 2006; **536**: 182-191
61 **Leclercq IA**, Lebrun VA, Stärkel P, Horsmans YJ. Intrahepatic insulin resistance in a murine model of steatohepatitis: effect of PPARgamma agonist pioglitazone. *Lab Invest* 2007; **87**: 56-65
62 **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
63 **Zou Y**, Li J, Lu C, Wang J, Ge J, Huang Y, Zhang L, Wang Y. High-fat emulsion-induced rat model of nonalcoholic steatohepatitis. *Life Sci* 2006; **79**: 1100-1107
64 **Ito M**, Suzuki J, Tsujioka S, Sasaki M, Gomori A, Shirakura T, Hirose H, Ito M, Ishihara A, Iwaasa H, Kanatani A. Longitudinal analysis of murine steatohepatitis model induced by chronic exposure to high-fat diet. *Hepatol Res* 2007; **37**: 50-57
65 **Deng QG**, She H, Cheng JH, French SW, Koop DR, Xiong S, Tsukamoto H. Steatohepatitis induced by intragastric overfeeding in mice. *Hepatology* 2005; **42**: 905-914
66 **Romestaing C**, Piquet MA, Bedu E, Rouleau V, Dautresme M, Hourmand-Ollivier I, Filippi C, Duchamp C, Sibille B. Long term highly saturated fat diet does not induce NASH in Wistar rats. *Nutr Metab (Lond)* 2007; **4**: 4
67 **Nishikawa S**, Yasoshima A, Doi K, Nakayama H, Uetsuka K. Involvement of sex, strain and age factors in high fat diet-induced obesity in C57BL/6J and BALB/cA mice. *Exp Anim* 2007; **56**: 263-272
68 **Ogasawara M**, Hirose A, Ono M, Aritake K, Nozaki Y, Takahashi M, Okamoto N, Sakamoto S, Iwasaki S, Asanuma T, Taniguchi T, Urade Y, Onishi S, Saibara T, Oben JA. A novel and comprehensive mouse model of human non-alcoholic steatohepatitis with the full range of dysmetabolic and histological abnormalities induced by gold thioglucose and a high-fat diet. *Liver Int* 2011; **31**: 542-551
69 **Matsuzawa N**, Takamura T, Kurita S, Misu H, Ota T, Ando H, Yokoyama M, Honda M, Zen Y, Nakanuma Y, Miyamoto K, Kaneko S. Lipid-induced oxidative stress causes steatohepatitis in mice fed an atherogenic diet. *Hepatology* 2007; **46**: 1392-1403
70 **Kawasaki T**, Igarashi K, Koeda T, Sugimoto K, Nakagawa K, Hayashi S, Yamaji R, Inui H, Fukusato T, Yamanouchi T. Rats fed fructose-enriched diets have characteristics of nonalcoholic hepatic steatosis. *J Nutr* 2009; **139**: 2067-2071
71 **Ackerman Z**, Oron-Herman M, Grozovski M, Rosenthal T, Pappo O, Link G, Sela BA. Fructose-induced fatty liver disease: hepatic effects of blood pressure and plasma triglyceride reduction. *Hypertension* 2005; **45**: 1012-1018
72 **Armutcu F**, Coskun O, Gürel A, Kanter M, Can M, Ucar F, Unalacak M. Thymosin alpha 1 attenuates lipid peroxidation and improves fructose-induced steatohepatitis in rats. *Clin Biochem* 2005; **38**: 540-547
73 **Spruss A**, Kanuri G, Wagnerberger S, Haub S, Bischoff SC, Bergheim I. Toll-like receptor 4 is involved in the development of fructose-induced hepatic steatosis in mice. *Hepatology* 2009; **50**: 1094-1104
74 **Kohli R**, Kirby M, Xanthakos SA, Softic S, Feldstein AE, Saxena V, Tang PH, Miles L, Miles MV, Balistreri WF, Woods SC, Seeley RJ. High-fructose, medium chain trans fat diet induces liver fibrosis and elevates plasma coenzyme Q9 in a

novel murine model of obesity and nonalcoholic steatohepatitis. *Hepatology* 2010; **52**: 934-944

75 **Nomura K**, Yamanouchi T. The role of fructose-enriched diets in mechanisms of nonalcoholic fatty liver disease. *J Nutr Biochem* 2012; **23**: 203-208

76 **Ip E**, Farrell GC, Robertson G, Hall P, Kirsch R, Leclercq I. Central role of PPARalpha-dependent hepatic lipid turnover in dietary steatohepatitis in mice. *Hepatology* 2003; **38**: 123-132

77 **Kashireddy PV**, Rao MS. Lack of peroxisome proliferator-activated receptor alpha in mice enhances methionine and choline deficient diet-induced steatohepatitis. *Hepatol Res* 2004; **30**: 104-110

78 **Carmiel-Haggai M**, Cederbaum AI, Nieto N. A high-fat diet leads to the progression of non-alcoholic fatty liver disease in obese rats. *FASEB J* 2005; **19**: 136-138

79 **Arsov T**, Larter CZ, Nolan CJ, Petrovsky N, Goodnow CC, Teoh NC, Yeh MM, Farrell GC. Adaptive failure to high-fat diet characterizes steatohepatitis in Alms1 mutant mice. *Biochem Biophys Res Commun* 2006; **342**: 1152-1159

80 **Wouters K**, van Gorp PJ, Bieghs V, Gijbels MJ, Duimel H, Lütjohann D, Kerksiek A, van Kruchten R, Maeda N, Staels B, van Bilsen M, Shiri-Sverdlov R, Hofker MH. Dietary cholesterol, rather than liver steatosis, leads to hepatic inflammation in hyperlipidemic mouse models of nonalcoholic steatohepatitis. *Hepatology* 2008; **48**: 474-486

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REVIEW

Gastric stimulation for weight loss

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Abstract

The prevalence of obesity is growing to epidemic proportions, and there is clearly a need for minimally invasive therapies with few adverse effects that allow for sustained weight loss. Behavior and lifestyle therapy are safe treatments for obesity in the short term, but the durability of the weight loss is limited. Although promising obesity drugs are in development, the currently available drugs lack efficacy or have unacceptable side effects. Surgery leads to long-term weight loss, but it is associated with morbidity and mortality. Gastric electrical stimulation (GES) has received increasing attention as a potential tool for treating obesity and gastrointestinal dysmotility disorders. GES is a promising, minimally invasive, safe, and effective method for treating obesity. External gastric pacing is aimed at alteration of the motility of the gastrointestinal tract in a way that will alter absorption due to alteration of transit time. In addition, data from animal models and preliminary data from human trials suggest a role for the gut-brain axis in the mechanism of GES. This may involve alteration of secretion of hormones associated with hunger or satiety. Patient selection for gastric stimulation therapy seems to be an important determinant of the treatment's outcome. Here, we review the current status, potential mechanisms of action, and possible future applications of gastric stimulation for obesity.

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Key words: Obesity; Gastric stimulation; Gastric motility; External pacing; Gastric pacing; Intestinal pacing

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INTRODUCTION

Obesity is a major public health challenge. The evolving concepts of how nutrient excess and inflammation modulate metabolism provide new opportunities for strategies to correct the damaging health consequences of obesity. The traditional approaches of caloric restriction, exercise, and behavioral therapies can each produce substantial weight loss, but it is not sustained in the majority of patients. The pharmacological agents that are currently licensed for weight loss produce only modest results and may have unwanted side effects. Clearly, a safe, effective, and durable treatment for obesity is needed.

The role of the gastrointestinal tract in regulating energy balance is now well recognized, and the expanding understanding of gut endocrinology and electrical signaling has produced a number of potentially fruitful avenues for developing obesity therapies.

In the last few years, gastric stimulation has gained attention as a possible new approach for treating obesity. Gastric electrical stimulation (GES) is a new method for invoking gastric contractions using a microprocessor con-

troller. The effect of this long-term gastric stimulation on food intake and body weight was first studied in pigs[1,2]. Pigs treated for eight months with electrical antral stimulation showed a net decrease of food intake[1]. In other studies, a significant decrease in food intake has been noted with gastric stimulation in dogs, and a significant decrease in fat absorption has been reported in rats[3-6].

Because GES has shown promising results in several animal models, the technique has been further refined for obesity treatment in humans. In 1995, the technique of an implantable gastric pacing device was studied in 24 morbidly obese patients. The results demonstrated the safety of the method and revealed changes in eating habits that resulted in reduced food intake and weight loss[7]. In a subsequent human study, there was a reported increase in meal-related satiety and enhanced inter-meal satiety during the treatment[8]. From this information, gastric pacing was hypothesized to facilitate weight loss by enhancing neuroendocrine satiety mechanisms.

Here, we review some of the techniques and the potential mechanisms associated with using gastric stimulation for treating obesity.

ROLE OF THE GUT-BRAIN AXIS IN REGULATING APPETITE

Food intake is influenced by emotional factors, social cues, and learned behavior. These influences overlay highly conserved systems within the brain that sense and integrate signals reflecting overall energy stores, recent energy intake and the presence of specific classes of nutrients.

Signals from the gut are important for controlling appetite and for regulating energy balance and glucose homeostasis. The active brain-gut axis sets the basis for options to stimulate different brain areas or neural connections between the brain and the gut to induce sensations of satiety.

There are several potential means by which the gut and the brain are associated. Some involve connections from the gut to hormones and from hormones to the nervous system. The gut continuously sends information to the brain regarding the quality and quantity of ingested food. These signals are not only important for satiation and meal termination but also for the appetitive phase of eating behavior[9]. By acting on the brainstem and hypothalamus, this flow of sensory information from the gut to the brain generates the feeling of satisfaction that is observed after a satiating meal[9].

The vagus nerve (VN) contributes to the bidirectional communications between the gastrointestinal tract and central nervous system (CNS)[10]. Afferent neurons of the VN are important targets of gut hormones, particularly the hormones involved in controlling food intake. Vagovagal reflexes are involved in feeding homeostasis, making neuromodulation an attractive method for managing obesity[10].

Ziomber *et al*[10] have described the parameters of vagal neuromodulation required to decrease food intake in rats and cause a resulting reduction in body mass. Rats with solenoid electrodes placed on the left VN significantly decreased their food intake, weight gain and serum leptin concentrations. Another study has shown the suppressive effects of vagal nerve stimulation (VNS) on long-term feeding regulation in rats[11]. VNS has been shown to increase vagal afferent satiety signals, leading to reduced food intake and decreased weight gain[11]. Chronic microchip vagal stimulation significantly decreases epididymal fat pad weight and meal size in VNS rats, which causes decreased weight gain.

In addition to the VN, several other areas in the brain are involved in regulating gastrointestinal motility and satiety. The central nervous system regulates the homeostasis between nutrient intake and body reserves by sensing nutrient levels, integrating the information, and regulating energy intake and/or energy expenditure[12]. Grill *et al*[13] have summarized several potential mechanisms for connections between the gut and the brain. The nucleus tractus solitarius (NTS) and circuits within the hindbrain mediate the intake-inhibition effects of gastrointestinal (GI) signals. Short-term eating behavior is also controlled by the hindbrain. The NTS receives inputs from VN afferent neurons, while the area postrema is a target for circulating factors, such as amylin and glucagon-like peptide 1 (GLP-1)[14]. Classical studies have shown that when the higher inputs are surgically interrupted, the hindbrain can continue to regulate food intake in response to peripheral signals[15].

GASTRIC HORMONES ASSOCIATED WITH HUNGER AND SATIETY

The mechanisms that modulate gut-related satiety signals are now being recognized. The apparent importance of alterations in the gut hormonal milieu caused by surgical interventions in the GI tract has led to new surgical approaches and devices. Interventions (such as gastric pacing) that modulate gut-related satiety signals may offer approaches for curbing the threat of obesity to human health. The enteroendocrine cells of the GI tract act as a luminal surveillance system, responding to either the presence or absence of food in the gut lumen[16]. Their secretion products regulate the course of digestion and determine the delivery of nutrients to the gut by controlling food intake[16]. The gut is the source of numerous peptides, many of which can alter appetite. These peptides have numerous targets, including gastrointestinal exocrine glands, smooth muscles, afferent nerve terminals, and the brain[17].

GLP-1

This incretin hormone is secreted from the lower gut in response to food intake stimulates insulin secretion and

inhibits gastric emptying[18,19]. Following an oral glucose load, insulin secretion is enhanced when compared with an intravenous glucose infusion in the presence of similar plasma glucose concentrations[20]. This phenomenon is referred to as the incretin effect. In patients with type 2 diabetes mellitus (T2DM), however, the incretin effect is significantly impaired and postprandial GLP-1 secretion is diminished[20]. Because both the incretin effect and postprandial GLP-1 concentrations are markedly reduced in T2DM patients[21], the first clinical studies addressed the question of whether raising GLP-1 levels by exogenous administration of the incretin hormone could help to restore normal glucose regulation. However, GLP-1 has a plasma half-life of just a few minutes because it is readily degraded by the ubiquitous enzyme dipeptidyl peptidase-IV. Although the beneficial clinical effects of GLP-1 are evident, the need to continuously infuse it have hampered its broad clinical applications. Therefore, incretin mimetic agents, such as exenatide and dipeptidyl peptidase-IV inhibitors, are used to treat patients with T2DM[22]. Some studies have indicated that this treatment also results in weight loss[23,24]. Because of its short half-life, it has been shown that peripheral administration of GLP-1 lowers postprandial glucose dysregulation by inhibiting glucagon secretion. Beta-cell function is also improved, along with increased satiety and decreased food intake, which then results in weight loss. GLP-1 stimulates insulin secretion, which also delays gastric emptying[25-27]. Obese subjects have less GLP-1 release after a meal, which may lead to less inter-meal satiety when compared with lean subjects[28]. Low postprandial levels of GLP-1 in obese children are responsible for excessive ingestion of food and decreased inhibition of gastric emptying, both of which result in obesity[29]. The ability of Roux-en-Y gastric bypass surgery to increase GLP-1 levels has been reported[30]. In gastric pacing, up to 40% of excess body weight can be lost after 2 years of treatment[31]. A limited study in morbidly obese patients has shown that gastric pacing is associated with decreased peripheral levels of GLP-1. This effect correlates with decreased food intake and the resulting weight loss. From these results, a mechanistic role for the stimulation of vagal afferents has also been propounded[8].

Leptin

The nervous system regulates energy balance at the whole-body level by constantly adjusting energy intake, expenditure, and storage[32,33]. The *ob* gene encodes the protein leptin, which is an adipose tissue-derived circulating hormone. Since the identification of *ob* as the first example of human monogenic obesity, it has become clear that leptin plays a key role in controlling mammalian food intake and body fat stores[34,35]. A leptin deficiency in mice homozygous for a mutant *ob* gene (*ob*/*ob* mice) causes morbid obesity and diabetes. Leptin replacement leads to decreased food intake, normalized glucose homeostasis and increased energy expenditure[36-39]. Leptin induces transcriptional changes for several genes *via* the JAK/STAT3 pathway, and rapid changes in cellular activity and membrane potential may underlie the acute actions of leptin[40]. Leptin resistance limits its utility in ordinary obesity, as reflected by the increased levels of leptin in obese subjects relative to their increased body fat[41]. Therefore, while the actions of leptin in peripheral tissues have been identified, studies in genetically modified mice have demonstrated that leptin action in the CNS is sufficient to regulate body weight, feeding, energy expenditure, and glucose metabolism[42-44]. The effect of leptin on metabolism is supported by neurons distributed in the hypothalamus, midbrain, and brainstem. It has been suggested that morphological changes progressively develop in the brain during obesity[45-47], and further inquiry into the cellular mechanisms linking obesity, neural plasticity, and food cravings are needed. Certain gut peptides (e.g., ghrelin) act in an additive manner with leptin to regulate energy balance[48], which has led to the development of combination therapies to enhance leptin sensitivity in obese states. Roux-en-Y gastric bypass in obese patients is associated with a decrease in leptin levels after only minimal changes in BMI have occurred[49]. Gastric pacing in morbidly obese patients results in significant weight loss that correlates with a decrease in leptin levels[8].

Peptide YY

The gut hormone peptide YY (PYY) belongs to a family of peptides that includes pancreatic polypeptide and neuropeptide Y (NPY). It is secreted by the L cells of the lower intestine after ingesting a meal and is released into the circulation[50], where it exists in two endogenous forms: PYY_{1-36} and PYY_{3-36}[51]. PYY_{1-36} is the major form of PYY in the fasting state[51]. The latter form is produced by the action of the dipeptidyl peptidase-IV enzyme in response to food intake[52]. Between 1% and 10% of PYY is also found in the esophagus, stomach, duodenum and jejunum[53]. PYY exerts its action through NPY receptors. PYY inhibits gastric motility and increases water and electrolyte absorption in the colon[54].

Endogenous PYY may be involved in the long-term regulation of body weight. PYY enhances a reduction in hunger and food intake[55]. Peripheral administration of PYY_{1-36} decreases food intake in rodents. PYY_{3-36} also markedly inhibits food intake in rodents[56,57] In humans, an intravenous infusion of physiological levels of PYY_{3-36} reduces caloric intake in both normal weight[56] and obese subjects[58]. This effect is not achieved exclusively by affecting energy intake; there is evidence that PYY may have some effects on energy expenditure and lipid metabolism[59]. PYY levels are higher in rats treated with solenoid electrodes placed on the left VN than in untreated controls[10]. PYY may also be associated with the rapid improvement in carbohydrate homoeostasis observed after bypass surgery. This improvement is secondary to an increase in insulin sensitivity rather than an increase in insulin secretion, which occurs later[60]. Increases in

the secretion of GLP-1 and PYY are involved in the disappearance of hypertriglyceridemia and decreases in the levels of circulating fatty acids[60]. Some studies have reported that Roux-en-Y gastric bypass surgery increases PYY levels[30].

Cholecystokinin

Cholecystokinin (CCK) is the major hormone responsible for gallbladder contraction and pancreatic enzyme secretion. Like other gastrointestinal hormones, CCK is produced in discrete endocrine cells that line the mucosa of the small intestine. CCK stimulates vagal afferent neuron discharge and controls the expression of G-protein coupled receptors and peptide neurotransmitters in these neurons[16,61]. A gatekeeper function is attributed to CCK because its presence or absence influences the capacity of vagal afferent neurons to respond to other neurohormonal signals[16]. CCK reduces food intake by exerting an effect on the CCK-1 receptors residing in the VN[62].

During fasting, plasma CCK concentrations are low and vagal afferent neurons express cannabinoid CB1 and melanin concentrating hormone (MCH)-1 receptors, which stimulate food intake[16]. Post-prandial release of CCK down-regulates the expression of both receptors and stimulates the expression of Y2 receptors in neurons projecting to the stomach. In fasting, there is increased expression in the neurons of the appetite-stimulating neuropeptide transmitter MCH, along with decreased expression of the satiety-peptide cocaine and amphetamine-regulated transcript (CART). The secretion of CCK decreases the expression of MCH and increases the expression of CART. At low plasma concentrations of CCK, vagal afferent neurons exhibit increased capacity for appetite-stimulation, while post-prandial concentrations of CCK lead to enhanced capacity for satiety signaling[16].

CCK antagonists induce hunger, leading to larger meals sizes. CCK also delays the rate at which food empties from the stomach[62]. CCK expression can be altered by prolonged gastric stimulation. Following 14 d of GES, the number of CCK-immunoreactive neurons in the hippocampus increased compared with a control group[63]. In another study performed on morbidly obese patients treated with gastric pacing, the gastric pacing resulted in decreased CCK levels[8].

Ghrelin

Ghrelin was the first identified circulating hunger hormone[64]. In contrast to the GI peptide hormones (such as GLP-1, PYY and others) that increase satiety through CNS-mediated pathways[65,66], the gastric hormone ghrelin stimulates hunger through a different CNS-mediated pathway[65]. Ghrelin is produced primarily by cells in the oxyntic glands of the stomach and intestines[67] and is secreted into the bloodstream. Ghrelin is a potent stimulator of growth hormone (GH) secretion and is the only circulatory hormone known to potently enhance feeding and weight gain and to regulate energy homeostasis following central and systemic administration. When administered either peripherally or centrally to rodents, ghrelin increases food intake and body weight[68-70] and stimulates gastric motility and acid secretion[71]. In humans, there is a pre-prandial rise in plasma ghrelin levels[72,73]. When administered to rodents at supra-physiological doses, ghrelin is able to activate hypothalamic neuropeptide Y agouti-related protein neurons and increase both food intake and body weight. Ghrelin participates in meal initiation. When administered either peripherally or centrally to rodents, ghrelin rapidly increases food intake and body weight[48]. Therapeutic intervention with ghrelin in catabolic situations enhances food intake and increases gastric emptying and nutrient storage. These effects, coupled with an increase in GH, link nutrient partitioning with growth and repair processes. Ghrelin-based compounds may have therapeutic utility for treating the malnutrition and wasting that are induced by various sub-acute and chronic disorders. Conversely, compounds that inhibit ghrelin action may be useful for preventing or treating components of the metabolic syndrome, such as obesity, impaired lipid metabolism and insulin resistance.

Peripheral blood ghrelin levels were studied one month prior to gastric pacing, one month following implantation, and six months after activation of the electrical stimulation. Ghrelin levels were decreased significantly in response to food intake at all these evaluations. After activating the pacemaker, ghrelin levels were significantly increased above their levels prior to activation. The weight loss was significantly correlated with the increased ghrelin levels[74]. A decrease in the number of ghrelin-immunoreactive neurons in the hypothalamic paraventricular nucleus and in the supraoptic nucleus has been reported after a short period of gastric stimulation in rats[63]. In Roux-en-Y gastric bypass surgery, the total fasting plasma ghrelin levels were nearly identical between the subjects and the matched controls[30].

METHODS OF GASTRIC STIMULATION FOR THE TREATMENT OF OBESITY

Many aspects of cell functioning are controlled by intrinsic or extrinsic electrical activity. In the normal physiological state, an action potential is generated and then causes a cellular response. When electrical signals are applied externally, they increase the rate at which this response occurs, which is called electrical pacing. Nevertheless, if the electrical signal is applied during the refractory period of the normal action potential, it does not increase the response rate. However, it has been shown in several studies that application of the electrical signal is still able to change the biochemistry of the cell, which leads to greatly increased responses to the next normal action potential[75]. Stimulating the stomach, the sub-diaphragmatic sympathetics, the vagal nerve (with or without unilateral vagotomy), and the intestines are some of the pacing approaches that have been used for treating obesity[76].

Gastric pacing with short pulse width and high frequency for the treatment of obesity

Dilatation of the stomach by food ingestion sends afferent signals through the VN to the CNS and increases satiety, thus regulating food intake[77,78-80]. Because of the many neuro-hormonal functions of the stomach and the modification of these hormones by food ingestion, modifying the function of the stomach has been hypothesized to be a potential mechanism for treating obesity.

Several methods have been used to electrically stimulate the stomachs of obese patients[76]. Changes in the intensity of the stimulus, the duration of the pacing, and the anatomical site of stimulation determine the effect of the treatment. The responses to gastric pacing are dependent on the stimulus intensity, with shorter duration stimuli being sufficient when the impulse strength is increased. The antrum can be paced to significantly higher frequencies than the more proximal gastric regions.

GES is a method for invoking gastric contractions. The main effect of the technique was originally thought to be longer retention of food in the stomach that induces early satiety and diminishes food intake. The first clinical use of gastric pacing in the early 1990s was preceded by an exploration of gastric electrical physiology in the 1980s[81] Human GES for obesity began in 1995. There have now been more than 500 subjects treated for obesity with gastric pacing. With proper screening, the weight loss can be in the range of 40% of the excess body weight[76,82].

GES uses an electrical device called a gastric pacemaker to provide mild electrical stimulation to the lower abdominal nerves. Utilizing minimally-invasive surgical techniques, the gastric pacemaker is placed subcutaneously in the abdomen. It is intended to induce early satiety through electrical stimulation of the gastric wall. Several gastric stimulation protocols have demonstrated their weight-reduction efficacy in animal obesity models and in patients with morbid obesity. The implantable gastric stimulator (IGS) induces stomach expansion *via* electrical stimulation of the VN. This type of stimulation induces neurobiological responses by using brain circuits that lead to decreased food intake[83].

IGS has been used for the treatment of obesity[84]. IGS reduces appetite and increases satiety. Its efficacy is attributed to its inhibitory effects on gastric motility and its direct effects on the central nervous system and hormones related to satiety and/or appetite[84]. Chronic gastric stimulation impairs intrinsic gastric myoelectrical activity in the fed state, induces gastric distention in the fasting state and inhibits postprandial antral contractions[84]. The impairment of gastric myoelectrical activity and contractions are associated with impaired digestion and emptying of the stomach, which leads to early satiety and reduced food intake. The induction of gastric distention in the fasting state results in activation of stretch receptors, causing satiety[84]. Modulation of neuronal activities and the release of certain hormones in response to an IGS may also explain the reduction in appetite and increase in satiety.

The first studies of GES as a treatment for obesity employed the Transcend™ Implantable Gastric Stimulator. In 2002, Favretti and colleagues showed that the Transcend device was safe and effective at inducing and maintaining weight loss in 20 morbidly obese patients[85]. The pacemaker was implanted laparoscopically below the pes anserinus, 3 cm from the edge of the lesser gastric curvature and 6 cm from the pylorus. The lead was fixed within a tunnel in the gastric muscular wall by a suture, and gastroscopy was performed to ensure that the lead did not perforate the stomach wall. The operative time was less than an hour, and the stimulator was activated 30 d after its implantation. The stimulus parameters were an amplitude of 10 mA, a pulse width of 208 ms, and a frequency of 40 Hz with 2 s on and 3 s off[85]. The Transcend device blocks vagal efferents and delays gastric emptying, leading to a 40% loss of excess body weight[76]. The pacemaker delivers 2 s pulse trains with a 40-100 Hz frequency, 3-10 mA of current, and a short pulse duration of 0.18-0.4 ms, with intervening 3 s periods of no stimulation[7,86]. In another study, two electrode positions along the lesser curvature of the stomach were used, with the low insertions 6 cm proximal to the pylorus and the high insertions just distal to the esophageal-gastric junction. Short-term weight loss has been achieved in a subset of these patients[86]. The clinical response is most often defined by the excess weight lost (EWL) in these studies; however, improvements in symptoms, quality of life and nutritional status have also been reported by most open-labeled studies[87].

Gastric pacing with long pulse width and low frequency for obesity

Other types of stimuli have been tested in animal models and in humans. In dogs, retrograde stimulation through two electrode pairs positioned proximal to the pylorus for 8 s at a frequency of 50 Hz and a voltage of 16 V with intervening periods of no stimulation elicits retrograde contractions, reduces food intake and promotes weight reduction[88]. In another animal study, electrical stimulation has been shown to reduce food intake and increase gastric volume[89]. Studies in dogs using a pulse width in milliseconds at a frequency of six cycles per minute induced gastric relaxation when the pacing occurred in the proximal stomach but not when it occurred in the distal stomach[90]. These studies demonstrated the ability to inhibit gastric contractions and delay gastric emptying[90,91]. Temporary retrograde pacing with a mucosal electrode placed endoscopically on the greater curvature of the stomach, 5 cm above the pylorus, was performed in 12 normal volunteers. The gastric slow waves were entrained at nine cycles per minute, and the symptoms of satiety, bloating, discomfort, and nausea were linearly correlated with the energy stimulation in milliamps[92]. Later, 12 normal volunteers were studied for 3 d using temporary electrodes endoscopically placed

on the greater curvature stomach, 5 cm above the pylorus. Retrograde gastric pacing at nine cycles per minute resulted in retrograde propagation of electrical waves from the antrum. These waves disrupted the normal electrical waves that propagate distally and caused gastric hypomotility. Food intake decreased by 16% and gastric retention of solids increased by 15% during this retrograde pacing. These changes were accompanied by tolerable dyspeptic symptoms[92]. Another study in 12 normal volunteers using a stimulus intensity below the threshold that induces dyspepsia was able to delay gastric emptying, decrease food intake, and decrease water intake compared with sham controls. These results suggested the possibility of performing longer stimulations to treat obesity[93].

VNS for weight loss

The VN has a role in regulating hippocampal activity, and the hippocampus has a role in modulating eating behaviors[83]. Studies with the Transcend gastric pacemaker have suggested that blocking the efferent vagal impulses can reduce gastric tone, which is accompanied by slower gastric emptying, decreased food intake, and weight loss. A laparoscopically implanted electrical device that intermittently blocked both VNs near the esophageal-gastric junction led to EWL in obese patients[94]. A vagal blocking algorithm with a duration of 90-150 s was associated with greater EWL than were either shorter- or longer-duration algorithms[94]. An association was found between the number of 90-150 s algorithms delivered daily and greater EWL. In a study conducted in rabbits, the animals had a microchip implanted on the posterior vagus by laparotomy. Over a 4-wk period, body weight decreased by 12%, food intake decreased by 40%, and pulse rate decreased. Heart rate changes suggested stimulation of the afferent vagus in addition to blocking of the efferent vagus[95]. In another study, pigs were implanted with microchips on both vagal nerves by laparotomy. At an amplitude of 170 mV, a frequency of 1 Hz, and a 170-ms impulse duration, their food intake was decreased and their body weight gain was reduced with no observable side effects. Normogastria was reduced and tachygastria was increased, which is consistent with reduced efferent vagal transmission[96]. The human data on weight loss through VN stimulation comes from trials using VN stimulators for treating epilepsy. These patients had cervical vagal stimulation, which caused hoarseness, cough, throat pain, and dyspnea. Thirty-two patients were evaluated and 17 lost weight[97].

Stimulation during the electrical refractory period to treat obesity

Stimulating the gastric antrum in rats during the absolute refractory period increases the strength of gastric contractions and increases vagal afferent firing, similar to its effects on gastric distension[98]. Pyloric stimulation in dogs decreases food intake, decreases antral contractions, and delays gastric emptying[99]. The Tantalus system (MetaCure Ltd.) is implanted laparoscopically for gastric stimulation that does not exhibit malabsorptive or restrictive characteristics[100]. It was developed to electrically stimulate the gastric antral muscle immediately following the entrance of food into the stomach. It increases antral muscular contractions and delays gastric emptying by delivering stimulation during the absolute refractory period[76]. The Tantalus system has a pulse generator and three bipolar leads. Two pairs of electrodes are implanted in the gastric antrum and two pairs in the gastric fundus. The electrodes in the gastric fundus sense the beginning of a meal and signal the pulse generator to stimulate the antral electrodes during the absolute antral refractory period, which enhances spontaneous gastric contractions and sends a signal through the afferent vagus that the stomach is distended. The device applies gastric contractility modulation signals to the gastric antrum. The system is designed to automatically detect when eating begins and to only then deliver GES sessions using electrical pulses that are synchronized to the intrinsic antral slow waves[100,101]. This method involves surgical placement of three electrode pairs; one pair in the fundus detects food ingestion, and two pairs in the antrum detect the intrinsic slow waves and deliver stimuli in synchrony with these signals[102]. The gastric stimulation begins when food enters the stomach, so it is only delivered postprandially. The device is believed to work by increasing antral contractions. This stimulated phased motor activity enhances the satiety that is normally elicited by postprandial gastric distention[98]. The stimulation parameters that enhance the phased antral contractions include a frequency of 80 Hz, a pulse width of 1-2 s, and a current of 0.5-1 mA[98]. Because of its energy requirements, the device must be recharged weekly by an external charger.

A trial in 12 obese subjects demonstrated that the fundal electrodes were able to sense the start of a meal > 75% of the time. The subjects lost 10 kg over 20 wk, and 9 subjects who continued till week 52 lost an additional 7 kg, for a total of 30.5% of their excess body weight lost[102]. In a European multicenter, open-label study, thirteen T2DM obese patients were laparoscopically implanted with the Tantalus device. The thirteen subjects that completed 3 mo of treatment showed a significant reduction in weight that was accompanied by glycemic improvement[100]. In the eleven patients that completed 6 mo of therapy, HbA1c was significantly reduced. However, the improvement in glucose control did not correlate with weight loss[101]. The data support the hypothesis that GES can improve glucose metabolism and induce weight loss in obese diabetic patients[103].

Intestinal electrical pacing for obesity

Intestinal electrical stimulation (IES) may have promising applications for treating motility disorders associated with altered intestinal contractile activity. However, recent studies have also revealed possible applications of intestinal electrical stimulation in obesity treatment[104]. IES applied to the duodenum reduces postprandial blood

glucose levels by modulating gastric emptying and the intestinal flow rate[105]. Both vagal and extra-vagal pathways are involved in the modulatory effects of IES on the central neurons of the satiety center[106]. Electrical stimulation of the stomach, intestine, or colon with long pulses has an inhibitory effect on gastric tone[107]. IES has been reported to alter intestinal slow waves, contractions and transit, effects that are mediated by both vagal and adrenergic pathways[108]. Duodenal stimulation in 12 healthy human volunteers did not induce dyspepsia, but it did reduce water intake and slowed gastric emptying[43]. Intestinal stimulation in dogs using seven sequential electrodes with a frequency of 24 cycles per minute, a pulse duration of 50 ms, and a pulse amplitude of 1-3 mA entrains the intestinal pacesetter. This treatment is effective at stimulating intestinal transit, even in the face of fat in the distal small intestine[109]. Intestinal stimulation in rats accelerates intestinal transit and reduces fat absorption[110]. Clinical trials of intestinal pacing in humans have yet to be reported.

Some potential mechanisms for the effect of gastric pacing in obesity

Obesity is the result of an imbalance between nutrient consumption, absorption, and energy expenditure[111]. GI motility regulates the rates at which nutrients are processed and absorbed and participates in controlling appetite and satiety *via* mechanical and neuro-hormonal pathways. The relatively extensive information on gastric pacing for motility disorders has shed light on the possible mechanisms of this treatment's beneficial effects on obesity. The effects of pacing may depend on the stimulus parameters and stimulation sites. Both the entrainment of intrinsic gastric electrical activity, eliciting propagating contractions and reducing symptomatology in patients with gastroparesis and reducing appetite and food intake in morbid obesity were suggested[112]. Additionally, gastric stimulation parameters have extra-gastrointestinal effects, including altering systemic hormonal and autonomic neural activity and modulating afferent nerve pathways projecting to the central nervous system[112].

Obesity that is induced by hypothalamic damage is associated with increased vagal tone, increased insulin secretion, increased food intake, and weight gain[113]. Performing a vagotomy below the diaphragm reverses the obesity caused by hypothalamic damage in rodents. Because trials have shown that placing a gastric pacemaker is the safest and simplest surgical treatment for morbid obesity, there has been considerable interest in defining the mechanisms by which it works[31]. Obesity seems to be associated with efferent vagal stimulation, which is inhibited by the Transcend pacemaker that is currently used. Short pulse widths and high-frequency stimulation induces gastric distention, inhibits postprandial antral contractions, and slows gastric emptying, which then leads to early satiety, reduced food intake and weight loss[114]. Cholinergic vagal efferents increase gastric tone, while nitric oxide pathways decrease it[115]. Gastric stimulation blocks the efferent vagal pathway and releases nitric oxide pathways from inhibition, resulting in gastric dilatation[84,116,117].The gastric slow waves are inhibited postprandially, which may also contribute to the delayed gastric emptying and promotion of satiety[118].

The decreased levels of GI hormones may be more compatible with depressed vagal tone than with vagal electrical stimulation of the pes anserinus area (the spreading zone of the vagal branches in the lesser curvature of the stomach). However, it is also possible that gastric pacing leads to parasympathetic hyperstimulation and the depletion of stored peptides, which may explain the reduced plasma levels of certain gastrointestinal hormones[8]. GES may also affect several brain areas associated with satiety and hunger, and may exert an effect on the hormonal gut-brain axis. Information on the current metabolic state is transmitted to the appetite control centers of the brain by a diverse array of signals, such as VN activity and metabolic "feedback" factors that are derived from the pituitary gland, adipose tissue, stomach, intestines, pancreas and muscle[12]. These signals act directly on the neurons located in the arcuate nucleus of the medio-basal hypothalamus, a key integration and hunger (orexigenic) and satiety (anorexigenic) control center of the brain[12].

CONCLUSION

Obesity is an epidemic disease that is increasing in prevalence and is associated with a rising incidence of diabetes. Behavior and lifestyle therapy are safe and effective treatments for obesity in the short term, but the durability of the weight loss is limited. Although promising obesity drugs are in development, the currently available drugs lack efficacy or have unacceptable side effects. Surgery leads to long-term weight loss, but it is associated with morbidity and mortality. GES is a promising, minimally invasive, safe, and effective method for treating obesity. Patient selection for gastric stimulation therapy seems to be an important determinant of the treatment's outcome[86,119-121]. Several approaches with different physiologic mechanisms are in various stages of development. The use of GES as an obesity treatment should produce exciting new developments in the foreseeable future.

REFERENCES

1 **Cigaina V**, Saggioro A, Rigo V, Pinato G, Ischai S. Long-term Effects of Gastric Pacing to Reduce Feed Intake in Swine. *Obes Surg* 1996; **6**: 250-253

2 **Cigaina V**, Pinato G, Rigo V, Bevilacqua M, Ferraro F, Ischia S, Saggioro A. Gastric Peristalsis Control by Mono Situ Electrical Stimulation: a Preliminary Study. *Obes Surg* 1996; **6**: 247-249

3 **Le Blanc-Louvry I**, Guerre F, Songné B, Ducrotté P. Gastric stimulation: influence of electrical parameters on gastric emptying in control and diabetic rats. *BMC Surg* 2002; **2**: 5

4 **Zhang J**, Xu X, Chen JD. Chronic tachygastrial electrical stimulation reduces food intake in dogs. *Obesity* (*Silver*

Spring) 2007; **15**: 330-339
5 **Xu L**, Sun X, Lu J, Tang M, Chen JD. Effects of gastric electric stimulation on gastric distention responsive neurons and expressions of CCK in rodent hippocampus. *Obesity* (*Silver Spring*) 2008; **16**: 951-957
6 **Zhang J**, Tang M, Chen JD. Gastric electrical stimulation for obesity: the need for a new device using wider pulses. *Obesity* (*Silver Spring*) 2009; **17**: 474-480
7 **Cigaina V**. Gastric pacing as therapy for morbid obesity: preliminary results. *Obes Surg* 2002; **12** Suppl 1: 12S-16S
8 **Cigaina V**, Hirschberg AL. Gastric pacing for morbid obesity: plasma levels of gastrointestinal peptides and leptin. *Obes Res* 2003; **11**: 1456-1462
9 **Berthoud HR**. Vagal and hormonal gut-brain communication: from satiation to satisfaction. *Neurogastroenterol Motil* 2008; **20** Suppl 1: 64-72
10 **Ziomber A**, Juszczak K, Kaszuba-Zwoinska J, Machowska A, Zaraska K, Gil K, Thor P. Magnetically induced vagus nerve stimulation and feeding behavior in rats. *J Physiol Pharmacol* 2009; **60**: 71-77
11 **Gil K**, Bugajski A, Kurnik M, Zaraska W, Thor P. Physiological and morphological effects of long-term vagal stimulation in diet induced obesity in rats. *J Physiol Pharmacol* 2009; **60** Suppl 3: 61-66
12 **Roche JR**, Blache D, Kay JK, Miller DR, Sheahan AJ, Miller DW. Neuroendocrine and physiological regulation of intake with particular reference to domesticated ruminant animals. *Nutr Res Rev* 2008; **21**: 207-234
13 **Grill HJ**, Hayes MR. The nucleus tractus solitarius: a portal for visceral afferent signal processing, energy status assessment and integration of their combined effects on food intake. *Int J Obes* (*Lond*) 2009; **33** Suppl 1: S11-S15
14 **Yamamoto H**, Kishi T, Lee CE, Choi BJ, Fang H, Hollenberg AN, Drucker DJ, Elmquist JK. Glucagon-like peptide-1-responsive catecholamine neurons in the area postrema link peripheral glucagon-like peptide-1 with central autonomic control sites. *J Neurosci* 2003; **23**: 2939-2946
15 **Grill HJ**, Smith GP. Cholecystokinin decreases sucrose intake in chronic decerebrate rats. *Am J Physiol* 1988; **254**: R853-R856
16 **Dockray GJ**. Cholecystokinin and gut-brain signalling. *Regul Pept* 2009; **155**: 6-10
17 **Schubert ML**. Gastric secretion. *Curr Opin Gastroenterol* 2010; **26**: 598-603
18 **Holst JJ**. Glucagonlike peptide 1: a newly discovered gastrointestinal hormone. *Gastroenterology* 1994; **107**: 1848-1855
19 **Drucker DJ**. The biology of incretin hormones. *Cell Metab* 2006; **3**: 153-165
20 **Nauck M**, Stöckmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 1986; **29**: 46-52
21 **Vilsbøll T**, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 2001; **50**: 609-613
22 **Baggio LL**, Drucker DJ. Biology of incretins: GLP-1 and GIP. *Gastroenterology* 2007; **132**: 2131-2157
23 **Riddle MC**, Henry RR, Poon TH, Zhang B, Mac SM, Holcombe JH, Kim DD, Maggs DG. Exenatide elicits sustained glycaemic control and progressive reduction of body weight in patients with type 2 diabetes inadequately controlled by sulphonylureas with or without metformin. *Diabetes Metab Res Rev* 2006; **22**: 483-491
24 **Blonde L**, Klein EJ, Han J, Zhang B, Mac SM, Poon TH, Taylor KL, Trautmann ME, Kim DD, Kendall DM. Interim analysis of the effects of exenatide treatment on A1C, weight and cardiovascular risk factors over 82 weeks in 314 overweight patients with type 2 diabetes. *Diabetes Obes Metab* 2006; **8**: 436-447
25 **Nauck MA**, Niedereichholz U, Ettler R, Holst JJ, Orskov C, Ritzel R, Schmiegel WH. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol* 1997; **273**: E981-E988
26 **van Genugten RE**, van Raalte DH, Diamant M. Does glucagon-like peptide-1 receptor agonist therapy add value in the treatment of type 2 diabetes? Focus on exenatide. *Diabetes Res Clin Pract* 2009; **86** Suppl 1: S26-S34
27 **Näslund E**, Barkeling B, King N, Gutniak M, Blundell JE, Holst JJ, Rössner S, Hellström PM. Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. *Int J Obes Relat Metab Disord* 1999; **23**: 304-311
28 **Ranganath LR**, Beety JM, Morgan LM, Wright JW, Howland R, Marks V. Attenuated GLP-1 secretion in obesity: cause or consequence? *Gut* 1996; **38**: 916-919
29 **Tomasik P**, Sztefko K, Starzyk J. Entero-insular axis in children with simple obesity. *Pediatr Endocrinol Diabetes Metab* 2009; **15**: 63-69
30 **Korner J**, Bessler M, Cirilo LJ, Conwell IM, Daud A, Restuccia NL, Wardlaw SL. Effects of Roux-en-Y gastric bypass surgery on fasting and postprandial concentrations of plasma ghrelin, peptide YY, and insulin. *J Clin Endocrinol Metab* 2005; **90**: 359-365
31 **Shikora SA**. Implantable Gastric Stimulation - the surgical procedure: combining safety with simplicity. *Obes Surg* 2004; **14** Suppl 1: S9-S13
32 **Bray GA,** Bouchard C, James WPT. Handbook of obesity. In: Historical framework for the development of ideas about obesity. New York: Mercel Dekker, 1998: 1-29
33 **Zhang Y**, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; **372**: 425-432
34 **Frederich RC**, Löllmann B, Hamann A, Napolitano-Rosen A, Kahn BB, Lowell BB, Flier JS. Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. *J Clin Invest* 1995; **96**: 1658-1663
35 **Maffei M**, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1995; **1**: 1155-1161
36 **Campfield LA**, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 1995; **269**: 546-549
37 **Halaas JL**, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995; **269**: 543-546
38 **Pelleymounter MA**, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 1995; **269**: 540-543
39 **Chua SC**, Chung WK, Wu-Peng XS, Zhang Y, Liu SM, Tartaglia L, Leibel RL. Phenotypes of mouse diabetes and rat fatty due to mutations in the OB (leptin) receptor. *Science* 1996; **271**: 994-996
40 **Williams KW**, Scott MM, Elmquist JK. From observation to experimentation: leptin action in the mediobasal hypothalamus. *Am J Clin Nutr* 2009; **89**: 985S-990S
41 **Farooqi IS**, Keogh JM, Kamath S, Jones S, Gibson WT, Trussell R, Jebb SA, Lip GY, O'Rahilly S. Partial leptin deficiency and human adiposity. *Nature* 2001; **414**: 34-35
42 **Morton GJ**, Niswender KD, Rhodes CJ, Myers MG, Blevins JE, Baskin DG, Schwartz MW. Arcuate nucleus-specific leptin receptor gene therapy attenuates the obesity phenotype of Koletsky (fa(k)/fa(k)) rats. *Endocrinology* 2003; **144**: 2016-2024

43 **Coppari R**, Ichinose M, Lee CE, Pullen AE, Kenny CD, McGovern RA, Tang V, Liu SM, Ludwig T, Chua SC, Lowell BB, Elmquist JK. The hypothalamic arcuate nucleus: a key site for mediating leptin's effects on glucose homeostasis and locomotor activity. *Cell Metab* 2005; **1**: 63-72

44 **Cohen P**, Zhao C, Cai X, Montez JM, Rohani SC, Feinstein P, Mombaerts P, Friedman JM. Selective deletion of leptin receptor in neurons leads to obesity. *J Clin Invest* 2001; **108**: 1113-1121

45 **Bouret SG**, Draper SJ, Simerly RB. Trophic action of leptin on hypothalamic neurons that regulate feeding. *Science* 2004; **304**: 108-110

46 **Pinto S**, Roseberry AG, Liu H, Diano S, Shanabrough M, Cai X, Friedman JM, Horvath TL. Rapid rewiring of arcuate nucleus feeding circuits by leptin. *Science* 2004; **304**: 110-115

47 **Horvath TL**, Sarman B, García-Cáceres C, Enriori PJ, Sotonyi P, Shanabrough M, Borok E, Argente J, Chowen JA, Perez-Tilve D, Pfluger PT, Brönneke HS, Levin BE, Diano S, Cowley MA, Tschöp MH. Synaptic input organization of the melanocortin system predicts diet-induced hypothalamic reactive gliosis and obesity. *Proc Natl Acad Sci USA* 2010; **107**: 14875-14880

48 **Castañeda TR**, Tong J, Datta R, Culler M, Tschöp MH. Ghrelin in the regulation of body weight and metabolism. *Front Neuroendocrinol* 2010; **31**: 44-60

49 **Rubino F**, Gagner M, Gentileschi P, Kini S, Fukuyama S, Feng J, Diamond E. The early effect of the Roux-en-Y gastric bypass on hormones involved in body weight regulation and glucose metabolism. *Ann Surg* 2004; **240**: 236-242

50 **Adrian TE**, Ferri GL, Bacarese-Hamilton AJ, Fuessl HS, Polak JM, Bloom SR. Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* 1985; **89**: 1070-1077

51 **Grandt D**, Schimiczek M, Beglinger C, Layer P, Goebell H, Eysselein VE, Reeve JR. Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1-36 and PYY 3-36. *Regul Pept* 1994; **51**: 151-159

52 **Mentlein R**, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* 1993; **214**: 829-835

53 **Taylor IL**. Distribution and release of peptide YY in dog measured by specific radioimmunoassay. *Gastroenterology* 1985; **88**: 731-737

54 **Liu CD**, Aloia T, Adrian TE, Newton TR, Bilchik AJ, Zinner MJ, Ashley SW, McFadden DW. Peptide YY: a potential proabsorptive hormone for the treatment of malabsorptive disorders. *Am Surg* 1996; **62**: 232-236

55 **Sainsbury A**, Schwarzer C, Couzens M, Fetissov S, Furtinger S, Jenkins A, Cox HM, Sperk G, Hökfelt T, Herzog H. Important role of hypothalamic Y2 receptors in body weight regulation revealed in conditional knockout mice. *Proc Natl Acad Sci USA* 2002; **99**: 8938-8943

56 **Batterham RL**, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA, Cone RD, Bloom SR. Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* 2002; **418**: 650-654

57 **Challis BG**, Pinnock SB, Coll AP, Carter RN, Dickson SL, O'Rahilly S. Acute effects of PYY3-36 on food intake and hypothalamic neuropeptide expression in the mouse. *Biochem Biophys Res Commun* 2003; **311**: 915-919

58 **Batterham RL**, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, Bloom SR. Inhibition of food intake in obese subjects by peptide YY3-36. *N Engl J Med* 2003; **349**: 941-948

59 **Guo Y**, Ma L, Enriori PJ, Koska J, Franks PW, Brookshire T, Cowley MA, Salbe AD, Delparigi A, Tataranni PA. Physiological evidence for the involvement of peptide YY in the regulation of energy homeostasis in humans. *Obesity* (*Silver Spring*) 2006; **14**: 1562-1570

60 **Andreelli F**, Amouyal C, Magnan C, Mithieux G. What can bariatric surgery teach us about the pathophysiology of type 2 diabetes? *Diabetes Metab* 2009; **35**: 499-507

61 **Polak JM**, Bloom SR, Rayford PL, Pearse AG, Buchan AM, Thompson JC. Identification of cholecystokinin-secreting cells. *Lancet* 1975; **2**: 1016-1018

62 **Moran TH**, Kinzig KP. Gastrointestinal satiety signals II. Cholecystokinin. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G183-G188

63 **Liu S**, Tang M, Tao S, Chen JD. Central expressions of ghrelin and cholecystokinin in rats with gastric electrical stimulation. *Obes Surg* 2008; **18**: 109-114

64 **Kojima M**, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656-660

65 **Moran TH**. Gut peptide signaling in the controls of food intake. *Obesity* (*Silver Spring*) 2006; **14** Suppl 5: 250S-253S

66 **Young AA**, Jodka C, Pittner R, Parkes D, Gedulin BR. Dose-response for inhibition by amylin of cholecystokinin-stimulated secretion of amylase and lipase in rats. *Regul Pept* 2005; **130**: 19-26

67 **Date Y**, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000; **141**: 4255-4261

68 **Tschöp M**, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000; **407**: 908-913

69 **Nakazato M**, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature* 2001; **409**: 194-198

70 **Wren AM**, Small CJ, Ward HL, Murphy KG, Dakin CL, Taheri S, Kennedy AR, Roberts GH, Morgan DG, Ghatei MA, Bloom SR. The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. *Endocrinology* 2000; **141**: 4325-4328

71 **Asakawa A**, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 2001; **120**: 337-345

72 **Cummings DE**, Frayo RS, Marmonier C, Aubert R, Chapelot D. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *Am J Physiol Endocrinol Metab* 2004; **287**: E297-E304

73 **Cummings DE**, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001; **50**: 1714-1719

74 **Cigaina V**, Hirschberg AL. Plasma ghrelin and gastric pacing in morbidly obese patients. *Metabolism* 2007; **56**: 1017-1021

75 **Burkhoff D**, Shemer I, Felzen B, Shimizu J, Mika Y, Dickstein M, Prutchi D, Darvish N, Ben-Haim SA. Electric currents applied during the refractory period can modulate cardiac contractility in vitro and in vivo. *Heart Fail Rev* 2001; **6**: 27-34

76 **Greenway F**, Zheng J. Electrical stimulation as treatment for obesity and diabetes. *J Diabetes Sci Technol* 2007; **1**: 251-259

77 **Rolls BJ**, Roe LS, Meengs JS. Reductions in portion size and energy density of foods are additive and lead to sustained decreases in energy intake. *Am J Clin Nutr* 2006; **83**: 11-17

78 **Phillips RJ**, Powley TL. Gastric volume rather than nutrient content inhibits food intake. *Am J Physiol* 1996; **271**: R766-R769

79 **Jones KL**, Doran SM, Hveem K, Bartholomeusz FD, Morley JE, Sun WM, Chatterton BE, Horowitz M. Relation between postprandial satiation and antral area in normal subjects. *Am J Clin Nutr* 1997; **66**: 127-132
80 **Ladabaum U**, Koshy SS, Woods ML, Hooper FG, Owyang C, Hasler WL. Differential symptomatic and electrogastrographic effects of distal and proximal human gastric distension. *Am J Physiol* 1998; **275**: G418-G424
81 **Abell TL**, Kim CH, Malagelada JR. Idiopathic cyclic nausea and vomiting--a disorder of gastrointestinal motility? *Mayo Clin Proc* 1988; **63**: 1169-1175
82 **Cigaina V**. Long-term follow-up of gastric stimulation for obesity: the Mestre 8-year experience. *Obes Surg* 2004; **14** Suppl 1: S14-S22
83 **Wang GJ**, Yang J, Volkow ND, Telang F, Ma Y, Zhu W, Wong CT, Tomasi D, Thanos PK, Fowler JS. Gastric stimulation in obese subjects activates the hippocampus and other regions involved in brain reward circuitry. *Proc Natl Acad Sci USA* 2006; **103**: 15641-15645
84 **Chen J**. Mechanisms of action of the implantable gastric stimulator for obesity. *Obes Surg* 2004; **14** Suppl 1: S28-S32
85 **Favretti F**, De Luca M, Segato G, Busetto L, Ceoloni A, Magon A, Enzi G. Treatment of morbid obesity with the Transcend Implantable Gastric Stimulator (IGS): a prospective survey. *Obes Surg* 2004; **14**: 666-670
86 **D'Argent J**. Gastric electrical stimulation as therapy of morbid obesity: preliminary results from the French study. *Obes Surg* 2002; **12** Suppl 1: 21S-25S
87 **Soffer E**, Abell T, Lin Z, Lorincz A, McCallum R, Parkman H, Policker S, Ordog T. Review article: gastric electrical stimulation for gastroparesis--physiological foundations, technical aspects and clinical implications. *Aliment Pharmacol Ther* 2009; **30**: 681-694
88 **Neshev E**, Onen D, Jalilian E, Mintchev MP. Pre-pyloric neural electrical stimulation produces cholinergically-mediated reverse peristalsis in the acute canine model of microprocessor-invoked gastric motility for the treatment of obesity. *Obes Surg* 2006; **16**: 510-520
89 **Ouyang H**, Yin J, Chen JD. Gastric or intestinal electrical stimulation-induced increase in gastric volume is correlated with reduced food intake. *Scand J Gastroenterol* 2006; **41**: 1261-1266
90 **Xing JH**, Brody F, Brodsky J, Larive B, Ponsky J, Soffer E. Gastric electrical stimulation at proximal stomach induces gastric relaxation in dogs. *Neurogastroenterol Motil* 2003; **15**: 15-23
91 **Familoni BO**, Abell TL, Gan Z, Voeller G. Driving gastric electrical activity with electrical stimulation. *Ann Biomed Eng* 2005; **33**: 356-364
92 **Yao SK**, Ke MY, Wang ZF, Xu DB, Zhang YL. Visceral response to acute retrograde gastric electrical stimulation in healthy human. *World J Gastroenterol* 2005; **11**: 4541-4546
93 **Liu J**, Hou X, Song G, Cha H, Yang B, Chen JD. Gastric electrical stimulation using endoscopically placed mucosal electrodes reduces food intake in humans. *Am J Gastroenterol* 2006; **101**: 798-803
94 **Camilleri M**, Toouli J, Herrera MF, Kow L, Pantoja JP, Billington CJ, Tweden KS, Wilson RR, Moody FG. Selection of electrical algorithms to treat obesity with intermittent vagal block using an implantable medical device. *Surg Obes Relat Dis* 2009; **5**: 224-229; discussion 229-230
95 **Sobocki J**, Thor PJ, Uson J, Diaz-Guemes I, Lipinski M, Calles C, Pascual S. Microchip vagal pacing reduces food intake and body mass. *Hepatogastroenterology* 2001; **48**: 1783-1787
96 **Matyja A**, Thor PJ, Sobocki J, Laskiewicz J, Kekus J, Tuz R, Koczanowski J, Zaraska W. Effects of vagal pacing on food intake and body mass in pigs. *Folia Med Cracov* 2004; **45**: 55-62
97 **Burneo JG**, Faught E, Knowlton R, Morawetz R, Kuzniecky R. Weight loss associated with vagus nerve stimulation. *Neurology* 2002; **59**: 463-464
98 **Peles S**, Petersen J, Aviv R, Policker S, Abu-Hatoum O, Ben-Haim SA, Gutterman DD, Sengupta JN. Enhancement of antral contractions and vagal afferent signaling with synchronized electrical stimulation. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G577-G585
99 **Ding HG**, Wang BE, Zhao CH, Jia JD, Xu YL, Tang SZ. [Effects on intracellular Ca2+ and expression of L-type voltage-operated calcium channel protein in activated hepatic stellate cells stimulated by Chinese herbal compound 861]. *Zhonghua Gan Zang Bing Za Zhi* 2005; **13**: 922, 926
100 **Bohdjalian A**, Ludvik B, Guerci B, Bresler L, Renard E, Nocca D, Karnieli E, Assalia A, Prager R, Prager G. Improvement in glycemic control by gastric electrical stimulation (TANTALUS) in overweight subjects with type 2 diabetes. *Surg Endosc* 2009; **23**: 1955-1960
101 **Sanmiguel CP**, Conklin JL, Cunneen SA, Barnett P, Phillips EH, Kipnes M, Pilcher J, Soffer EE. Gastric electrical stimulation with the TANTALUS System in obese type 2 diabetes patients: effect on weight and glycemic control. *J Diabetes Sci Technol* 2009; **3**: 964-970
102 **Bohdjalian A**, Prager G, Aviv R, Policker S, Schindler K, Kretschmer S, Riener R, Zacherl J, Ludvik B. One-year experience with Tantalus: a new surgical approach to treat morbid obesity. *Obes Surg* 2006; **16**: 627-634
103 **Bohdjalian A**, Prager G, Rosak C, Weiner R, Jung R, Schramm M, Aviv R, Schindler K, Haddad W, Rosenthal N, Ludvik B. Improvement in glycemic control in morbidly obese type 2 diabetic subjects by gastric stimulation. *Obes Surg* 2009; **19**: 1221-1227
104 **Yin J**, Chen JD. Mechanisms and potential applications of intestinal electrical stimulation. *Dig Dis Sci* 2010; **55**: 1208-1220
105 **Khawaled R**, Blumen G, Fabricant G, Ben-Arie J, Shikora S. Intestinal electrical stimulation decreases postprandial blood glucose levels in rats. *Surg Obes Relat Dis* 2009; **5**: 692-697
106 **Zhang J**, Zhu H, Chen JD. Central neuronal mechanisms of intestinal electrical stimulation: effects on duodenum distention-responsive (DD-R) neurons in the VMH of rats. *Neurosci Lett* 2009; **457**: 27-31
107 **Xu X**, Lei Y, Chen JD. Effects and mechanisms of electrical stimulation of the stomach, duodenum, ileum, and colon on gastric tone in dogs. *Dig Dis Sci* 2010; **55**: 895-901
108 **Aelen P**, Jurkov A, Aulanier A, Mintchev MP. Pilot acute study of feedback-controlled retrograde peristalsis invoked by neural gastric electrical stimulation. *Physiol Meas* 2009; **30**: 309-322
109 **Chen JD**, Lin HC. Electrical pacing accelerates intestinal transit slowed by fat-induced ileal brake. *Dig Dis Sci* 2003; **48**: 251-256
110 **Sun Y**, Chen J. Intestinal electric stimulation decreases fat absorption in rats: therapeutic potential for obesity. *Obes Res* 2004; **12**: 1235-1242
111 **Gallagher TK**, Geoghegan JG, Baird AW, Winter DC. Implications of altered gastrointestinal motility in obesity. *Obes Surg* 2007; **17**: 1399-1407
112 **Hasler WL**. Methods of gastric electrical stimulation and pacing: a review of their benefits and mechanisms of action in gastroparesis and obesity. *Neurogastroenterol Motil* 2009; **21**: 229-243
113 **Bray GA**, Gallagher TF. Manifestations of hypothalamic obesity in man: a comprehensive investigation of eight patients and a reveiw of the literature. *Medicine* (*Baltimore*) 1975; **54**: 301-330
114 **Rogers PJ**. Eating habits and appetite control: a psychobiological perspective. *Proc Nutr Soc* 1999; **58**: 59-67
115 **Hermann GE**, Travagli RA, Rogers RC. Esophageal-gastric relaxation reflex in rat: dual control of peripheral nitrergic

and cholinergic transmission. *Am J Physiol Regul Integr Comp Physiol* 2006; **290**: R1570-R1576
116 **Lei Y**, Xing J, Chen JD. Effects and mechanisms of implantable gastric stimulation on gastric distention in conscious dogs. *Obes Surg* 2005; **15**: 528-533
117 **Zheng ZL**, Rogers RC, Travagli RA. Selective gastric projections of nitric oxide synthase-containing vagal brainstem neurons. *Neuroscience* 1999; **90**: 685-694
118 **Ouyang H**, Yin J, Chen JD. Therapeutic potential of gastric electrical stimulation for obesity and its possible mechanisms: a preliminary canine study. *Dig Dis Sci* 2003; **48**: 698-705
119 **Greenstein RJ**, Belachew M. Implantable gastric stimulation (IGS) as therapy for human morbid obesity: report from the 2001 IFSO symposium in Crete. *Obes Surg* 2002; **12** Suppl 1: 3S-5S
120 **Wolff S**, Pross M, Knippig C, Malfertheiner P, Lippert H. [Gastric pacing. A new method in obesity surgery]. *Chirurg* 2002; **73**: 700-703
121 **Miller KA**. Implantable electrical gastric stimulation to treat morbid obesity in the human: operative technique. *Obes Surg* 2002; **12** Suppl 1: 17S-20S

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ORIGINAL ARTICLE

Matrix metalloproteinase-9 contributes to parenchymal hemorrhage and necrosis in the remnant liver after extended hepatectomy in mice

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Abstract

AIM: To investigate the effect of matrix metalloproteinase-9 (MMP-9) on the remnant liver after massive hepatectomy in the mouse.

METHODS: Age-matched, C57BL/6 wild-type (WT), MMP-9(-/-), and tissue inhibitors of metalloproteinases (TIMP)-1(-/-) mice were used. The mice received 80%-partial hepatectomy (PH). Samples were obtained at 6 h after 80%-PH, and we used histology, immunohistochemical staining, western blotting analysis and zymography to investigate the effect of PH on MMP-9. The role of MMP-9 after PH was investigated using a monoclonal antibody and MMP inhibitor.

RESULTS: We examined the remnant liver 6 h after 80%-PH and found that MMP-9 deficiency attenuated the formation of hemorrhage and necrosis. There were significantly fewer and smaller hemorrhagic and necrotic lesions in MMP-9(-/-) remnant livers compared with WT and TIMP-1(-/-) livers ($P < 0.01$), with no difference between WT and TIMP-1(-/-) mice. Serum alanine aminotransaminase levels were significantly lower in MMP-9(-/-) mice compared with those in TIMP-1(-/-) mice (WT: 476 ± 83 IU/L, MMP-9(-/-): 392 ± 30 IU/L, TIMP-1(-/-): 673 ± 73 IU/L, $P < 0.01$). Western blotting and gelatin zymography demonstrated a lack of MMP-9 expression and activity in MMP-9(-/-) mice, which was in contrast to WT and TIMP-1(-/-) mice. No change in MMP-2 expression was observed in any of the study groups. Similar to MMP-9(-/-) mice, when WT mice were treated with MMP-9 monoclonal antibody or the synthetic inhibitor GM6001, hemorrhagic and necrotic lesions were significantly smaller and fewer than in control mice ($P < 0.05$). These results suggest that MMP-9 plays an important role in the development of parenchymal hemorrhage and necrosis in the small remnant liver.

CONCLUSION: Successful MMP-9 inhibition attenuates the formation of hemorrhage and necrosis and might

be a potential therapy to ameliorate liver injury after massive hepatectomy.

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Key words: Matrix metalloproteinase; Liver remnant; Hepatectomy; Liver failure; Necrosis

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INTRODUCTION

In the field of hepatobiliary surgery, liver resection is considered the standard treatment for primary liver tumor and colorectal liver metastases[1-3]. Major liver resections have been associated with an increased morbidity and mortality compared with more limited resections. In particular, the outcomes are worsened when a chronic underlying liver disease is present[4]. Recent studies have shown that the volume of the remnant liver is correlated with perioperative morbidity and mortality[5-7], and an insufficient hepatic remnant (IHR) after extended hepatectomy is a critical issue.

On the other hand, liver transplantation (LT) is the treatment of choice for end-stage liver diseases. However, in the LT field, donor shortage and small-for-size grafts (SFSGs) are critical issues. Although split-liver transplantation (SOLT) is promising, failure of SFSGs in adult LT recipients[8,9] and after extensive hepatectomy for metastatic cancer hinders its application[10]. SFSG failure results in postoperative coagulopathy, cholestasis, ascites, encephalopathy, and death[11,12]. The mechanisms of SFSG failure remain incompletely understood.

One mechanism that has been implicated in the early development of SFSG failure is the formation of parenchymal hemorrhage and necrosis[13,14]. Previous investigators used electron microscopic studies to demonstrate sinusoidal breakdown leading to hemorrhage and necrosis after chemically or endotoxin-induced liver failure[15-17], hepatic ischemia-reperfusion injury[18-19], extensive hepatectomy[18,20,21], and LT with SFSG or SOLT[22-25]. These findings suggested that although the inciting factors might have different causes, the pathogenesis of sinusoidal breakdown leading to hemorrhage and necrosis might be similar.

Matrix metalloproteinases (MMPs) comprise a family of zinc-dependent neutral proteases that can degrade the extracellular matrix and basement membrane. MMPs play significant roles in cellular regulation, cell-cell communication, and tumor progression[26,27]. MMP-2 and MMP-9 have been implicated in liver injury and remodeling. Knocking out MMP-9 and other MMPs attenuate liver injury associated with interferon treatment[28]. MMP-9 contributes to the pathogenesis of experimental acute liver failure[29]. Specifically, MMP-9 has been implicated in sinusoidal breakdown leading to extravasation of circulating cells and hemorrhage[14,30]. MMP-9 plays a pivotal role in ischemia-reperfusion injury[31-33]. When MMP-9 is blocked with a synthetic inhibitor or by MMP-9 gene deletion, it successfully reverses ischemia-reperfusion injury[34,35] and cold preservation-warm reperfusion injury after LT[35]. Collectively, these data show that MMP-9 is involved in sinusoidal injury in liver failure. However, the role of MMP-9 in the pathogenesis of failure in IHR or SFSGs is not well known.

We hypothesized that MMP-9 plays an important role in the development of liver parenchymal hemorrhage and necrosis in IHR/SFSG failure. Tissue inhibitors of metalloproteinases (TIMP)-1 are physiological tissue inhibitors of MMP-9, which increase MMP-9 activity when deleted[36,37]. We hypothesized that a deletion or decrease in MMP-9 has a beneficial effect after hepatectomy. In the current study, we examined this hypothesis in IHR after 80%-partial hepatectomy (PH) in wild-type (WT), MMP-9(-/-), and TIMP-1(-/-) mice. We also examined the efficacy of MMP-9 blockade with a monoclonal antibody and synthetic MMP-9 inhibitor.

MATERIALS AND METHODS

Animals

Male C57BL/6 mice (10-14 wk old) were obtained from Jackson Laboratory (Bar Harbor, ME), housed with a 12-h light/dark cycle, and given food and water *ad libitum*. MMP-9(-/-) mice, obtained from Dr. Robert Senior (Washington University, St. Louis, MO), and TIMP-1(-/-) mice, purchased from Jackson Laboratory, both with a C57BL/6 background, were bred in our facility. The study was institutionally approved in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals".

PH in mice

Our institutional procedures for PH and postoperative care have been described elsewhere in detail[38]. Left and right posterior and left and right anterior segments were resected for an 80%-PH, similar to that described in a previous report[39]. In brief, under general anesthesia, liver lobes were mobilized. A hemostatic clip (Teleflex Medical, Triangle Park, NC) was applied across the pedicle, and the liver lobes were removed, without any obstruction of portal flow and hepatic venous drainage[38]. Laparotomy alone was performed for sham operations. Before closure, 2 mL of warm saline was administered

intraperitoneally. Cephalexin (30 mg/kg) and buprenorphine (0.1 mg/kg) were given subcutaneously. Postoperatively, the mice were kept in a temperature-controlled environment with free access to food and water. All mice were sacrificed 6 h after 80%-PH. The remnant livers were harvested, and blood samples were collected. We chose the extended hepatectomy model in mice because we hypothesized that MMP-9 plays an important role in the development of liver parenchymal hemorrhage and necrosis in IHR/SFSG failure.

MMP-9 inhibition by anti-MMP-9 monoclonal antibody and the MMP inhibitor GM6001

WT mice received 3 mg/kg of anti-MMP-9 neutralizing monoclonal antibody intravenously (clone 6-6B; EMD Chemicals, Gibbstown, NJ) 1 h before 80%-PH (MMP-9 mAb, $n = 6$). Control mice received normal IgG (EMD, Gibbstown, NJ) (control IgG, $n = 6$). The broad-spectrum MMP-inhibitor GM6001 (Millipore, Billerica, MA) at a concentration of 100 mg/kg in 10% dimethyl sulfoxide (DMSO) was administrated intraperitoneally 2 h before 80%-PH ($n = 10$), and controls received DMSO only ($n = 10$). An inhibitor of MMP-9 itself may affect liver regeneration after PH. Therefore, for the inhibition of MMP-9, we employed two inhibitory methods (i.e., a monoclonal antibody and inhibitor) and used MMP-9(-/-) mice in this study.

Biochemical analysis

Serum levels of aspartate aminotransferase (AST) and alanine aminotransaminase (ALT) were determined by using a kinetic detection kit (Pointe Scientific, Inc, Canton, MI), and total bilirubin was determined by using the QuantiChrom™ Bilirubin Assay Kit (BioAssay Systems, Hayward, CA).

Western blotting analysis

Liver samples were homogenized in a buffer containing 10 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl, 1% Triton-X, 0.1% sodium dodecyl sulfate (SDS), 1 mmol/L ethylene diamine tetra-acetic acid (EDTA), 1 mmol/L ethylene glycol tetra-acetic acid, 1 mmol/L phenylmethyl-sulfonyl fluoride, and protease and phosphatase inhibitors. Homogenates were centrifuged at 105 000 × *g* for 1 h at 4 ℃. Supernatants were collected, and protein concentration was determined by bicinchoninic acid assay (Pierce, Rockford, IL). Forty micrograms of protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride membrane (Millipore, Bedford, MA). Membranes were blocked with 5% nonfat milk in Tris-buffered saline with Tween 20 [20 mmol/L Tris-buffered saline (pH 7.4), 500 mmol/L NaCl, and 0.05% Tween 20] and probed using the antibody for MMP-9 (R and D Systems, Minneapolis, MN), and were then incubated with peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) followed by enhanced chemi-luminescence (ECL) or ECL Plus reagent (Amersham Biosciences, Piscataway, NJ). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as control (Imgenex Corporation, San Diego, CA). Signals were quantified by using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Gelatin zymography

Liver homogenates were analyzed by gelatin zymography with affinity chromatography[40]. In brief, 400 μg of liver extract samples were incubated with 100 μL of gelatin-Sepharose 4B (GE Healthcare, Piscataway, NJ) and equilibrated buffer containing 50 mmol/L Tris-HCL pH 7.5, 150 mmol/L NaCl, 5 mmol/L $CaCl_2$, 0.02% Tween 20, and 10 mmol/L EDTA for 2 h at 4 ℃. After being washed three times, gelatin-Sepharose beads were resuspended in the same volume of 2 × zymography sample buffer (Bio-Rad Laboratories, Inc., Hercules, CA) and loaded onto a 10% SDS-PAGE gel containing 1 mg/mL of gelatin (Bio-Rad Laboratories, Inc.). After electrophoresis, the gel was washed twice for 30 min with 2.5% Triton X-100 for renaturing and then incubated in development buffer (Bio-Rad Laboratories, Inc.) for 20 h at 37 ℃. The gel was then fixed and stained with 0.5% Coomassie Blue R-250 (Bio-Rad Laboratories, Inc.) for 1 h and destained with 10% acetic acid in 40% methanol solution. Gelatinase zymography standards (Millipore, Billerica, MA) were used as a positive control.

Histology and immunohistochemical staining

Formalin-fixed, paraffin-embedded liver specimens in 5-μm sections were stained with hematoxylin and eosin. Immunohistochemical staining for CD11b (MAC-1) and CD68 was performed on frozen sections, and staining for desmin, myeloperoxidase, and MMP-9 was performed on paraffin-embedded sections after heat-induced antigen retrieval. We used 0.3% H_2O_2 to quench the endogenous peroxidase activity. An ABC kit (Vector Laboratories, Inc, Burlingame, CA) was used according to the manufacturer's instructions for HRP-based staining with 3,3' diaminobenzidine (DAB) tetrahydrochloride (Dako, Carpinteria, CA). Antibodies for MMP-9 [antigen affinity-purified, lyophilized from a 0.2 μm filtrated solution in phosphate buffered saline (PBS) with trehalose; AF909; R and D Systems, Minneapolis, MN], CD11b (clone M1/70, R and D Systems), CD68 (clone FA-11, Abcam Inc., Cambridge, MA), desmin (Abcam Inc.), and myeloperoxidase (Ab-1) (Thermo Scientific, Fremont, CA) were incubated for the primary reaction. Alexa Fluor 568 donkey anti-goat IgG (H + L), Alexa Fluor 488 donkey anti-rabbit IgG, and Alexa Fluor 488 donkey anti-rat antibody (Invitrogen, Carlsbad, CA) were used for secondary reactions.

Histological analysis

The HE-stained sections were scanned with a Scanscope XT system and analyzed with Aperio Imagescope software (Aperio Technologies, Inc., Vista, CA). Histologically positive areas were counted and measured by investigators blinded to the study group in three randomly

selected 3-mm^2 fields. DAB-positive staining of MMP-9 was calculated using the positive-pixel-count function of Imagescope software in 10 randomly selected fields. The value was quantified as the percentage of positive pixels per total pixels. The number of infiltrated neutrophils in the remnant liver was evaluated in five randomly captured 40 × high-power fields (hpf) with an Olympus BX50 fluorescence microscope (Olympus Optical, Tokyo, Japan).

In situ zymography

In situ gelatinolytic activity was determined using 20-μm cryostat sections for the EnzChek Gelatinase assay kit (Molecular Probes, Eugene, OR)[40]. Sections were incubated with 20 μg/mL of fluorescence-conjugated gelatin in reaction buffer (50 mmol/L Tris-HCl, 150 mmol/L NaCl, 5 mmol/L $CaCl_2$, and 0.2 mmol/L sodium azide) for 2 h at 37 ℃. After three washes with PBS, they were fixed in 4% paraformaldehyde. The sections were mounted with Vectashield (Vector Laboratories Inc., Burlingame, CA). Gelatinase activity was visualized using fluorescence microscopy (Olympus BX50; Olympus Optical, Tokyo, Japan).

Preliminary study

In our institution, 90%-PH (10% of the liver remnant with an omental segment), 80%-PH (20% of the liver remnant with right middle and omental segments) and 75%-PH (25% of the liver remnant with right middle and posterior segments) are available[38]. Initially, we performed 90%-PH in WT, MMP-9(-/-) and TIMP-1(-/-) mice, as a preliminary study. A survival study after 90%-PH was performed in each murine strain. Moreover, liver damage scores in HE staining[41], serum AST and ALT levels, and the ratios of terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling-positive nuclei/all nuclei per mm^2 and caspase 3-positive nuclei/all nuclei per mm^2 in immunohistological staining were assessed in serum or liver samples 6 h after 90%-PH in each murine strain. The results for each factor did not reach significant differences between WT and MMP-9(-/-) mice and between WT and TIMP-1(-/-) mice. Even in WT mice (control group), all mice received 90%-PH without any surgical issues but showed considerable damage and subsequently died in the early postoperative period. We therefore considered that 90%-PH is not suitable to investigate the effect of MMP-9 and TIMP-1 on the hepatic remnant after hepatectomy. To obtain relevant data according to clinical background[1-10], we finally employed 80%-PH in each strain for further experiments in this study. We decided to evaluate 80%-PH because mice could survive for a long time after surgery.

Statistical analysis

Data are presented as the mean ± SE. Statistical comparisons were performed using analysis of variance followed by a two-sample *t*-test with Bonferroni adjustment. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Parenchymal hepatic hemorrhage and necrosis occurs soon after 80%-PH

First, 80%-PH was performed in WT mice. Six hours after 80%-PH, the animals were killed and divided into two groups according to the status of parenchymal hemorrhage and necrosis in the remnant livers. Murine behavior in hepatic failure has been well described[42,43]. The animals with hemorrhage and necrosis were observed to be sick and inactive, whereas the mice without such injury were asymptomatic and active. As shown in Figure 1A and B, the groups consisted of mice with minimal or no hemorrhage and necrosis (group Ⅰ, n = 6) or those with multifocal hemorrhage and necrosis (group Ⅱ, n = 5). Group Ⅰ had a significantly greater area of necrosis (2.97% ± 0.92% *vs* 0.11% ± 0.08%, $P < 0.05$) (Figure 1C) and number of necrotic foci compared with group Ⅱ (3.60 ± 0.87 *vs* 0.26 ± 0.17, $P < 0.01$) (Figure 1D). Group Ⅱ had significantly higher AST (893 ± 72 IU/L *vs* 28 ± 12 IU/L, $P < 0.05$) (Figure 1E), ALT (744 ± 35 IU/L *vs* 32 ± 77 IU/L, $P < 0.05$) (Figure 1F) and total bilirubin levels than those in group Ⅰ (4.45 ± 0.63 mg/dL *vs* 1.41 ± 0.19 mg/dL, $P < 0.01$) (Figure 1G). These results suggested that group Ⅱ mice were in the process of liver failure, and these results are consistent with previous reports[13,44-46].

Focal and/or patchy necrosis is an important finding after hepatectomy[13,44]. Progressive necrosis is found from the early postoperative period[13,44-46]. We observed that parenchymal hemorrhage and necrosis occurred as early as 1 h after 80%-PH, and increased in number and size at 12 h and 24 h (data not shown), consistent with recent reports[13,44-46].

MMP-9 is upregulated in the remnant liver with hemorrhage and necrosis

MMP-9 has been implicated in acute liver failure[14,29]. We investigated MMP-9 expression in the remnant livers of both groups Ⅰ and Ⅱ 6 h after 80%-PH. Western blotting analysis demonstrated that MMP-9 was upregulated in group Ⅱ compared with group Ⅰ ($P < 0.01$) (Figure 2A and B). When we examined immunohistochemical staining for MMP-9, hepatocytes showed no expression of MMP-9. However, MMP-9 was mainly observed in round-shaped cells and in stellate-shaped cells (Figure 3A and B). To evaluate the association between necrosis and MMP-9, we compared the expression of MMP-9 in 10 randomly selected fields of 3-mm^2 in area with and without hemorrhage and necrosis. MMP-9 expression in the foci of hemorrhage and necrosis was higher than that in normal liver parenchyma ($P < 0.05$) (Figure 3C). These results suggest that MMP-9 expression is associated with the development of hemorrhage and necrosis in the remnant liver after 80%-PH, which is consistent with previous reports[14,29].

CD11b-positive neutrophils are the main source of MMP-9 in the remnant liver

To determine the source of upregulated MMP-9 in the

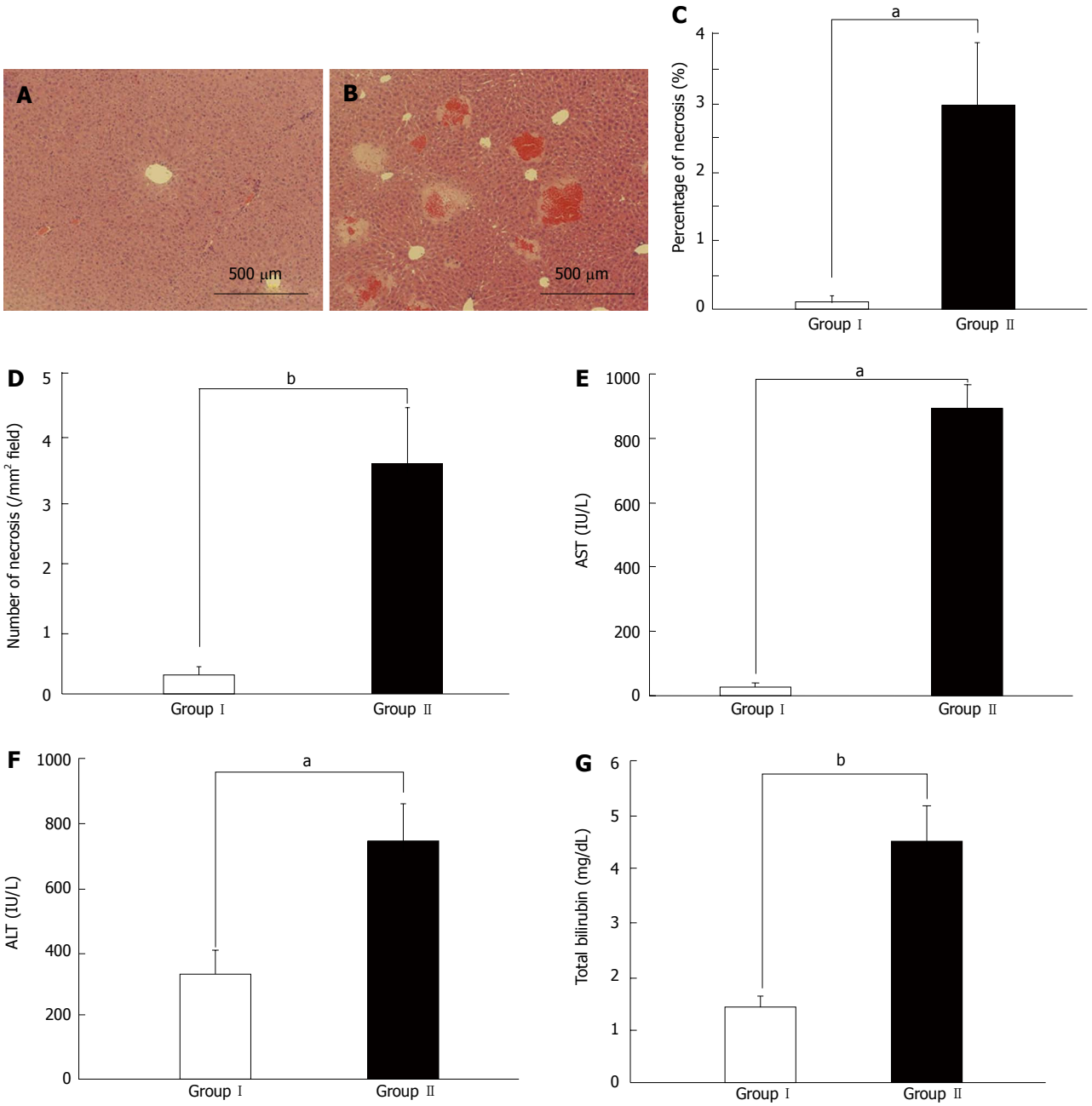

Figure 1 Histological and hematological differences between groups Ⅰ and Ⅱ at 6 h after 80%-partial hepatectomy. A: Histological findings in group Ⅰ mice; B: Representative images of remnant liver histology demonstrating significant multiple foci of hemorrhage and necrosis in group Ⅱ mice; C: Quantitation of the size (area percentage) of hemorrhage and necrosis: Significantly larger areas and more hemorrhagic and necrotic foci were observed in group Ⅱ mice than in group Ⅰ mice ($^aP < 0.05$); D: Quantitation of the number of foci of hemorrhage and necrosis per mm^2: Significantly larger areas and more hemorrhagic and necrotic foci were observed in group Ⅱ mice than in group Ⅰ mice ($^bP < 0.01$); E: Aspartate aminotransferase (AST) levels were significantly higher in group Ⅱ mice than those in group Ⅰ mice ($^aP < 0.05$); F: Alanine aminotransaminase (ALT) levels were significantly higher in group Ⅱ mice than those in group Ⅰ mice ($^aP < 0.05$); G: Total bilirubin levels were significantly higher in group Ⅱ mice than those in group Ⅰ mice ($^bP < 0.01$).

remnant liver, we performed double immunofluorescent analysis with MMP-9 and cell marker antibodies. CD68 and desmin were used for Kupffer cells and hepatic stellate cells, respectively. MMP-9 was not observed in CD68-positive Kupffer cells (Figure 4A-C). Weak immunoreactivity to MMP-9 was observed in desmin-positive hepatic stellate cells (Figure 4D-F). In contrast, we consistently found MMP-9 expression in CD11b-positive cells (Figure 4G-I). These results suggest that infiltrating neutrophils are the source of MMP-9 found in the foci of hemorrhage and necrosis in the remnant liver after 80%-PH, which is consistent with previous reports[28,35].

MMP-9 deletion reduces parenchymal hemorrhage and necrosis

To further confirm the role of MMP-9 in the development of liver hemorrhage and necrosis after 80%-PH, we performed liver resections in WT, MMP-9(-/-), and

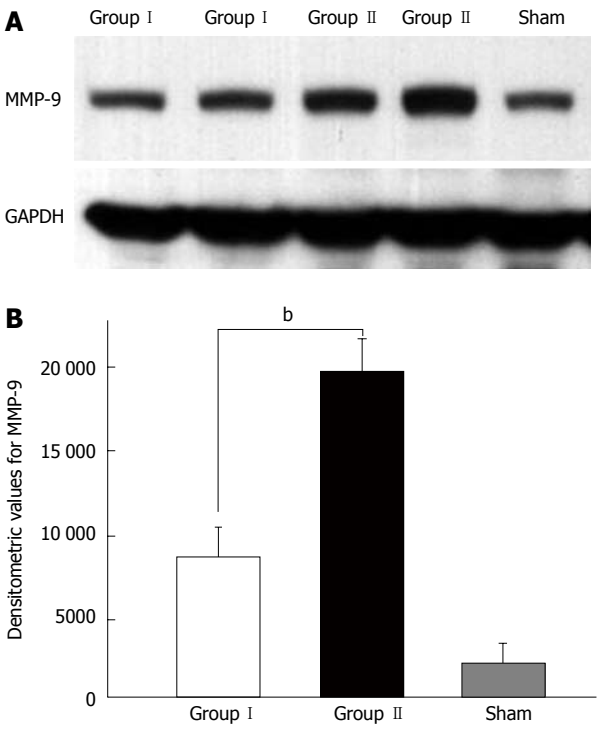

Figure 2 Western blotting analysis for matrix metalloproteinase-9 in remnant livers with (group Ⅱ) and without (group Ⅰ) foci of hemorrhage and necrosis after 80%-partial hepatectomy. Representative immunoblot (A) and histogram (B) show enhanced expression of matrix metalloproteinase (MMP)-9 protein in group Ⅱ mice compared with that in group Ⅰ mice and sham controls ($^bP < 0.01$).

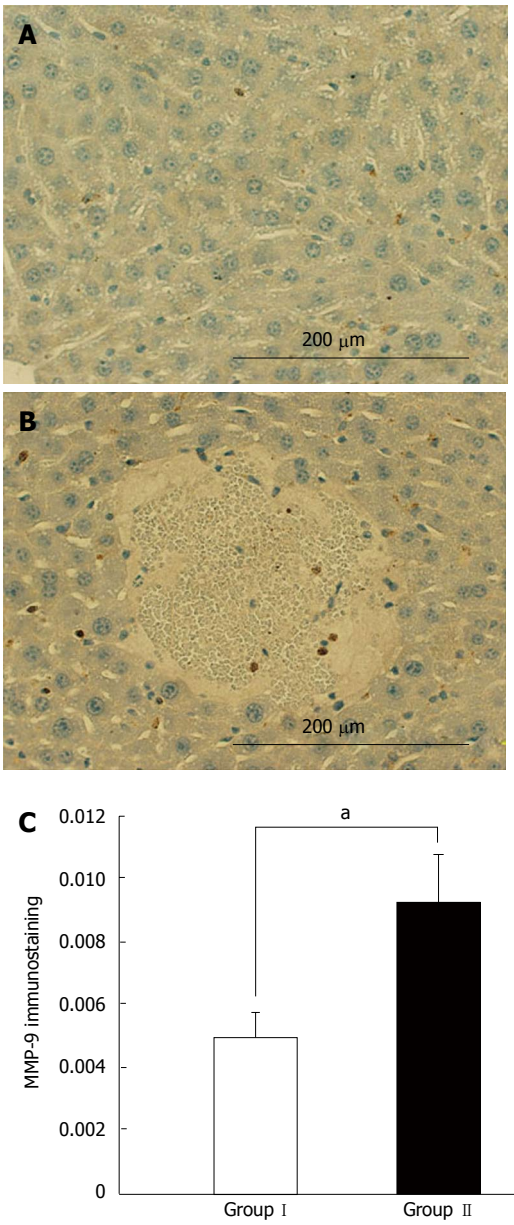

Figure 3 Immunohistochemical staining for matrix metalloproteinase-9 in the remnant liver. A: Representative images of matrix metalloproteinase (MMP)-9 staining in a non-necrotic area in group Ⅱ; B: Representative images of MMP-9 staining in a necrotic area in group Ⅱ; C: Histogram of MMP-9 expression as described in the material and methods section: Enhanced MMP-9 expression was observed in areas close to the focus of necrosis ($^aP < 0.05$).

TIMP-1(-/-) mice. TIMP-1 is a physiological tissue inhibitor of MMP-9, which increases MMP-9 activity when deleted[36,37]. We compared the size (area percentage) and the number of hemorrhagic and necrotic foci in the remnant livers. There were significantly fewer and smaller hemorrhagic and necrotic lesions in MMP-9(-/-) mice compared with WT and TIMP-1(-/-) mice (Figure 5A-C), (necrotic area: 0.49% ± 0.14% *vs* 1.74% ± 0.27% *vs* 1.82% ± 0.40%, $P < 0.01$, Figure 5D) (number of necrotic foci: 0.64 ± 0.18 *vs* 1.50 ± 0.21 *vs* 1.76 ± 0.39, $P < 0.05$, Figure 5E). There were no differences in the size and number of necrotic foci in WT and TIMP-1(-/-) animals.

Western blotting and gelatin zymography showed that MMP-9 expression and activity were present in WT and TIMP-1(-/-) mice but were absent in MMP-9(-/-) mice (Figure 6A and B). There was no difference in MMP-2 expression, a gelatinase, which is closely related to MMP-9, in the three groups of mice. In situ gelatin zymography showed significantly less MMP-9 activity in the remnant liver of MMP-9(-/-) mice compared with that in WT and TIMP-1(-/-) mice (Figure 6C-E). These results support our other findings that MMP-9 contributes to the development of parenchymal hemorrhage and necrosis in the remnant liver after 80%-PH.

MMP-9 deletion inhibits hepatic infiltration of neutrophils

To evaluate the effect of MMP-9 deficiency on hepatic neutrophil infiltration, we performed double immunofluorescent analysis of MMP-9 and myeloperoxidase, as previously described[47]. As shown in Figure 7A-C, we identified myeloperoxidase-positive neutrophils by their characteristic staining pattern in cytoplasmic azurophilic granules[48]. There were significantly fewer infiltrated neutrophils in MMP-9(-/-) mice than in WT and TIMP-1(-/-) mice (3.3 ± 0.4 *vs* 7.0 ± 1.1 *vs* 6.0 ± 0.8 per hpf, $P < 0.05$) (Figure 7D). These results suggest that MMP-9 deletion inhibits neutrophil infiltration of the remnant liver after massive hepatectomy.

Inhibition of MMP-9 with MMP-9 mAb or GM6001 ameliorates liver hemorrhage and necrosis

We performed an MMP-9 inhibition experiment with a

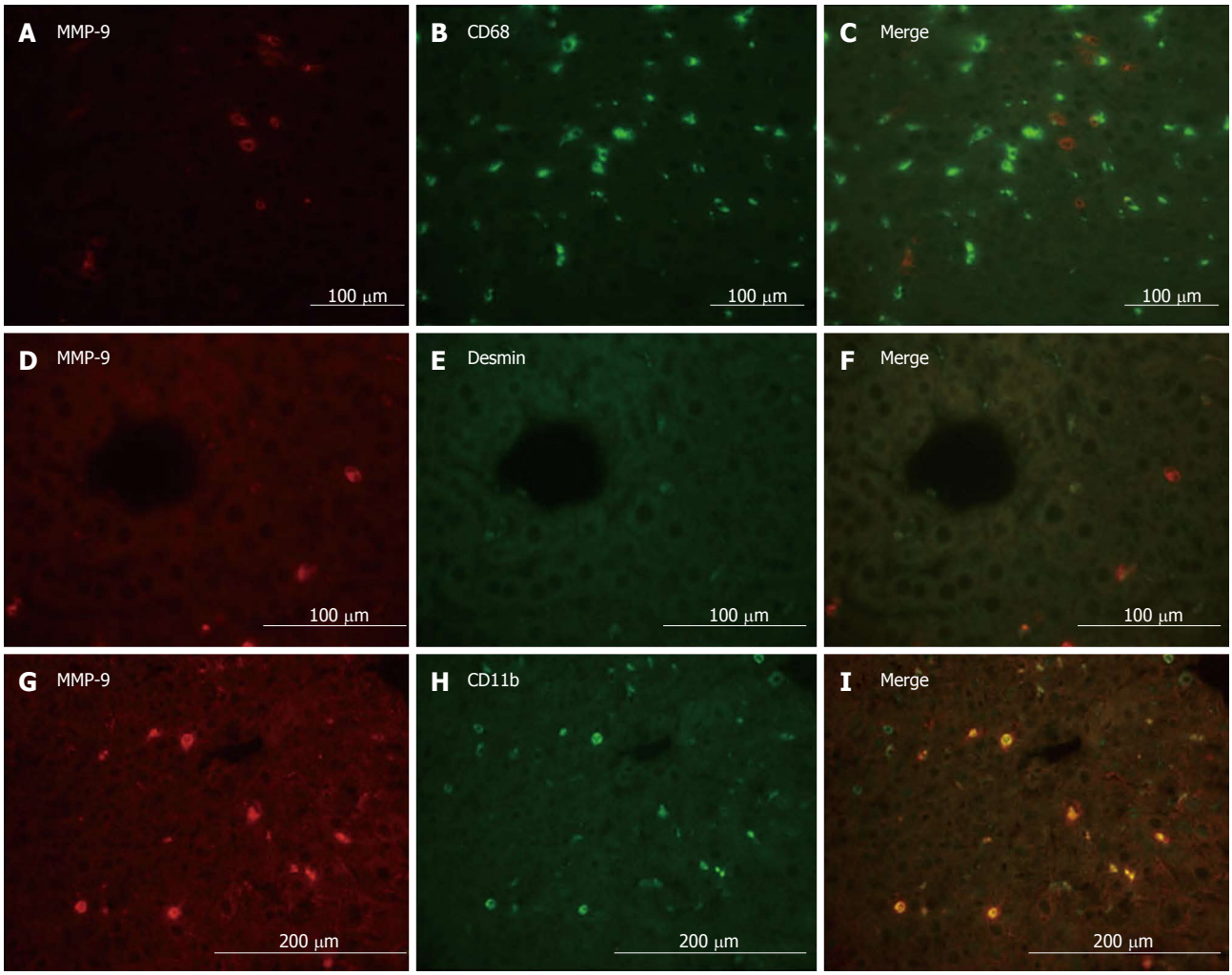

Figure 4 Co-localization analysis by dual immunofluorescence in the remnant liver after 80%-partial hepatectomy. Co-localization analysis by dual immunofluorescence in the remnant liver for (A) matrix metalloproteinase (MMP)-9 labeled in red (Alexa Fluor 568), (B) CD68 labeled in green (Alexa Fluor 488), and (C) both MMP-9 and CD68; for (D) MMP-9 labeled in red (Alexa Fluor 568), (E) desmin labeled in green (Alexa Fluor 488), and (F) both MMP-9 and desmin; and for (G) MMP-9 labeled in red (Alexa Fluor 568), (H) CD11b labeled in green (Alexa Fluor 488), and (I) both MMP-9 and CD11b. These co-localization results demonstrate that MMP-9 protein expression is mainly localized in CD11b-positive cells, and to a lesser extent in desmin-positive cells.

specific MMP-9 monoclonal antibody (MMP-9 mAb) and the broad-spectrum MMP inhibitor GM6001, as previously described[40]. There were significantly smaller and fewer necrotic areas in the remnant liver in mice treated with MMP-9 mAb than in control IgG mice (necrotic area: 0.17% ± 0.15% *vs* 1.81% ± 0.66%, $P < 0.05$; number of necrotic foci: 0.23 ± 0.16 *vs* 1.23 ± 0.44, $P < 0.05$) (Figure 8A and B). We showed inhibition of gelatinolytic activity with *in situ* gelatin zymography[40], which demonstrated significantly reduced MMP-9 activity in the MMP-9 mAb-treated group compared with that in the IgG-treated group (Figure 8C and D). Similarly, the number of infiltrated neutrophils was significantly lower in the group treated with MMP-9 mAb than that in the group treated with IgG (4.3 ± 1.1 *vs* 9.1 ± 1.9 per hpf, $P < 0.05$) (Figure 8E-G).

We observed similar results with GM6001. Treatment with GM6001 showed significant suppression of liver necrosis compared with the DMSO vehicle (necrotic area: 0.19% ± 0.13% *vs* 1.04% ± 0.36%, $P < 0.05$; number of necrotic foci: 0.35 ± 0.19 *vs* 1.12 ± 0.31, $P < 0.05$). Neutrophil infiltration was significantly suppressed in the GM6001 group compared with that in the DMSO group (5.5 ± 1.0 *vs* 9.2 ± 1.3 per hpf, $P < 0.05$). In situ zymographic analysis showed a significant reduction in gelatinolytic activity in the GM6001 group compared with that in the DMSO group (data not shown). Collectively, these results demonstrate that MMP-9 inhibition plays an important role in protecting mice from developing parenchymal hemorrhage and necrosis after 80%-PH.

DISCUSSION

IHR after extended liver resection or a SFSG after SOLT is prone to develop parenchymal hemorrhage and necrosis. Uncontrolled progression of the foci of hemorrhage and necrosis leads to liver failure. The role of MMP-9 in liver injury in the hepatic remnant has not been investigated. In this study, we investigated the role of MMP-9 in the development of hemorrhage and necrosis in IHR

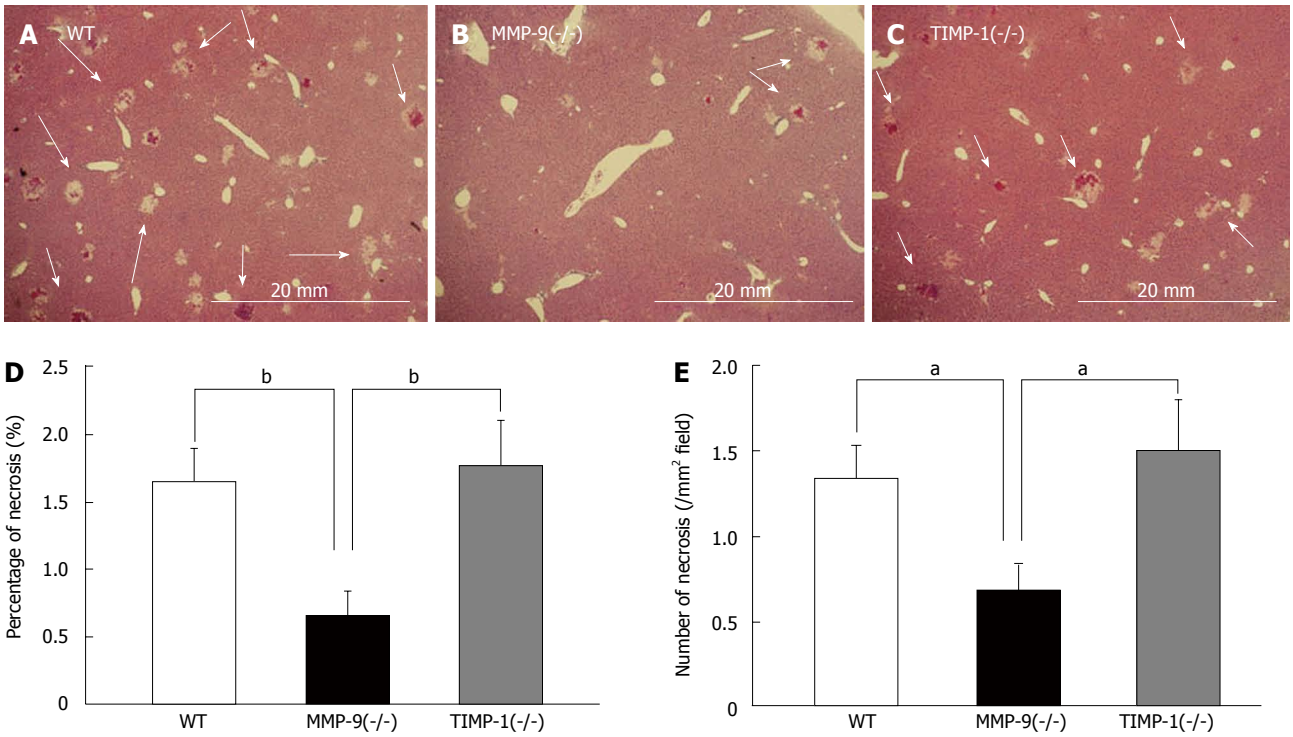

Figure 5 Histological analysis in each strain. Histological analysis between wild-type (WT, n = 15) (A), matrix metalloproteinase (MMP)-9(-/-) (n = 15) (B), and tissue inhibitors of metalloproteinases (TIMP)-1 (-/-) mice (n = 14) (C) at 6 h after 80%-partial hepatectomy (PH). Representative images of remnant liver histology at 6 h after 80%-PH in WT, MMP-9(-/-), and TIMP-1(-/-) mice. Foci of hemorrhage and necrosis are denoted by white arrows. The percentage of the necrotic area (D) and the number of necrotic foci per mm^2 (E) in remnant livers of study mice are shown. Significantly smaller and fewer necrotic foci in the remnant liver were observed in MMP-9(-/-) mice compared with WT and TIMP-1(-/-) mice ($^aP < 0.05$, $^bP < 0.01$).

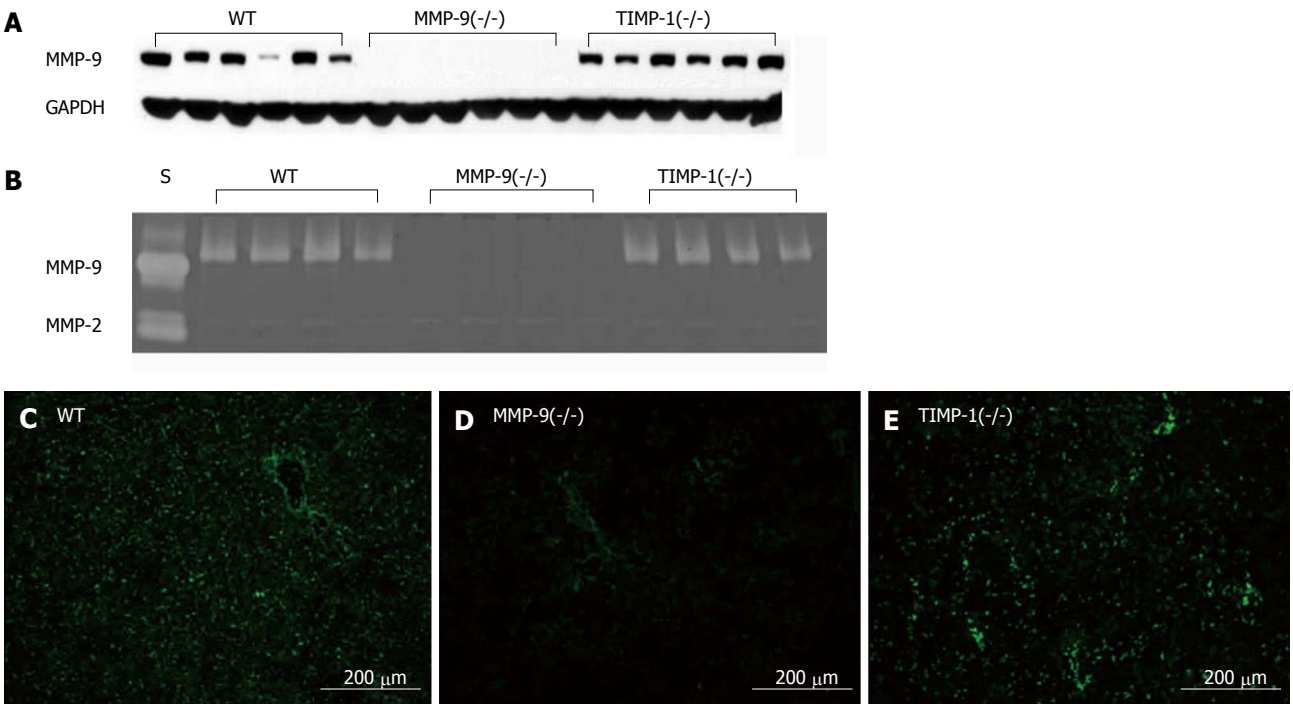

Figure 6 Expression and activity of matrix metalloproteinase-9. Expression and activity of matrix metalloproteinase (MMP)-9 were determined using (A) western blotting analysis for MMP-9 with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as a control, (B) polyacrylamide gel electrophoresis gelatin zymography with human MMP-9 and -2 standards (S), and *in situ* fluorescence gelatin zymography in the remnant livers of wild-type (WT), MMP-9(-/-), and tissue inhibitors of metalloproteinases (TIMP)-1(-/-) mice at 6 h after 80%-partial hepatectomy. We observed MMP-9 protein expression and activity in WT mice (C), but they were absent in MMP-9(-/-) mice (D). Enhancement of MMP-9 protein expression and activity in TIMP-1(-/-) mice was observed (E).

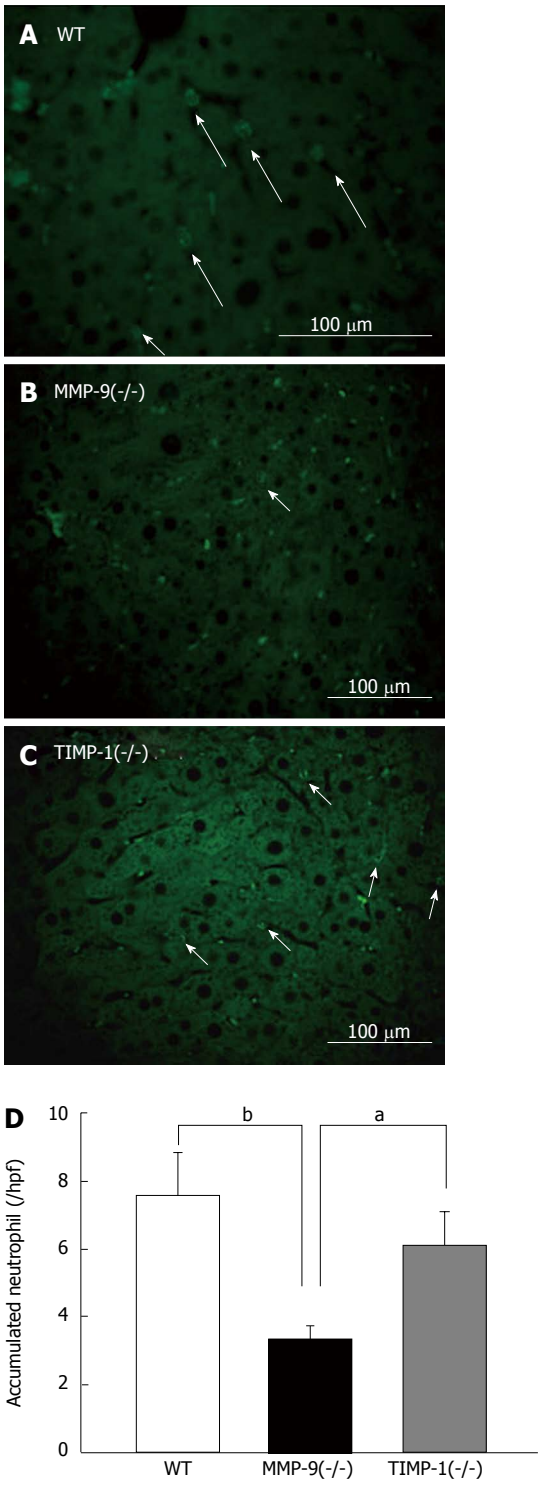

Figure 7 Immunohistochemical analysis of accumulated neutrophils with myeloperoxidase staining in remnant livers of wild-type, matrix metalloproteinase-9(-/-), and tissue inhibitors of metalloproteinases-1(-/-) mice at 6 h after 80%-partial hepatectomy. Representative images of myeloperoxidase staining, labeled in green (Alexa Fluor 488), of cytoplasmic azurophilic granules in neutrophils (arrows) in wild-type (WT) mice in remnant liver sections in (A) WT, (B) matrix metalloproteinase (MMP)-9(-/-), and (C) tissue inhibitors of metalloproteinases (TIMP)-1(-/-) mice. A histogram shows the number of accumulated neutrophils in the remnant liver (D). Significantly fewer neutrophils were observed in the remnant liver in MMP-9(-/-) mice than in WT and TIMP-1(-/-) mice ($^aP < 0.05$, $^bP < 0.01$).

after 80%-PH in mice. We demonstrated that in IHR 6 h postoperatively, MMP-9 was upregulated in infiltrating neutrophils associated with the foci of hemorrhage and necrosis. Blocking MMP-9 expression by using a synthetic inhibitor, a specific monoclonal antibody, or gene deletion significantly ameliorated the formation of parenchymal hemorrhage and necrosis.

In the current study, we found that the number and percentage of necrotic foci were decreased in MMP-9(-/-) mice compared with WT mice. While TIMP-1(-/-) mice appeared to show slightly increased necrosis, there were no statistical differences between WT and TIMP-1(-/-) mice. TIMP-1 is a physiological tissue inhibitor of MMP-9[36,37], and therefore, MMP-9 activity is increased under the absence of TIMP-1[36,37]. Our results demonstrated that a decrease or absence of MMP-9 prevented liver injury, and that a physiological inhibitor of MMP-9, i.e., TIMP-1, caused similar liver damage in WT mice with IHR after 80%-PH. One possible explanation for the similar damage between WT and TIMP-1(-/-) mice is that liver injury after 80%-PH is very severe in these mice.

In our study of IHR after 80%-PH in mice, we found that infiltrating neutrophils carry MMP-9, which appears to be responsible for the development of hemorrhage and necrosis. This is consistent with previous reports[14,28,35]. Wielockx and colleagues showed that MMPs, including MMP-9, play a central role in the destruction of sinusoidal integrity, resulting in hemorrhage and necrosis[28]. They speculated that MMPs could be derived from infiltrating neutrophils, resident hepatic stellate cells, and Kupffer cells. Recently, hepatic stellate cells have been shown to play a critical role in acute liver failure induced by lipopolysaccharide and beta-galactosamine or carbon tetrachloride by releasing MMP-9, which disrupts sinusoid integrity leading to sinusoidal collapse and liver failure[29]. Similarly, in hepatic warm ischemia-reperfusion injury, hepatic MMP-9 and MMP-2 induce sinusoidal injury in a manner that is independent of neutrophil cytotoxicity[35]. Together, these data suggest that decreasing MMP-9 expression is useful to reduce early sinusoidal injury in IHR and SFSGs.

Inhibition of MMP-9 appears to be a potential therapeutic option in ischemic liver injury. In a previous study in rats, 70% of the liver was rendered ischemic for 90 minutes followed by reperfusion; a phosphinic MMP inhibitor attenuated the liver injury and necrosis[49]. Gene deletion of MMP-9 mitigates liver injury after 90 minutes of warm ischemia and reperfusion in mice[34,35]. Inhibition of MMP-9 has been shown to protect rat livers from cold preservation-warm reperfusion injury[34,35]. Interestingly, after cold ischemic preservation and warm reperfusion, it was found that MMP-9 and MMP-2 were not of hepatocellular origin but from an extrahepatic source[34,35]. Therefore, collectively, these data support MMP-9 reduction as a potential pathway to improve parenchymal integrity and function in IHR after extended liver resection or a SFSG after SOLT.

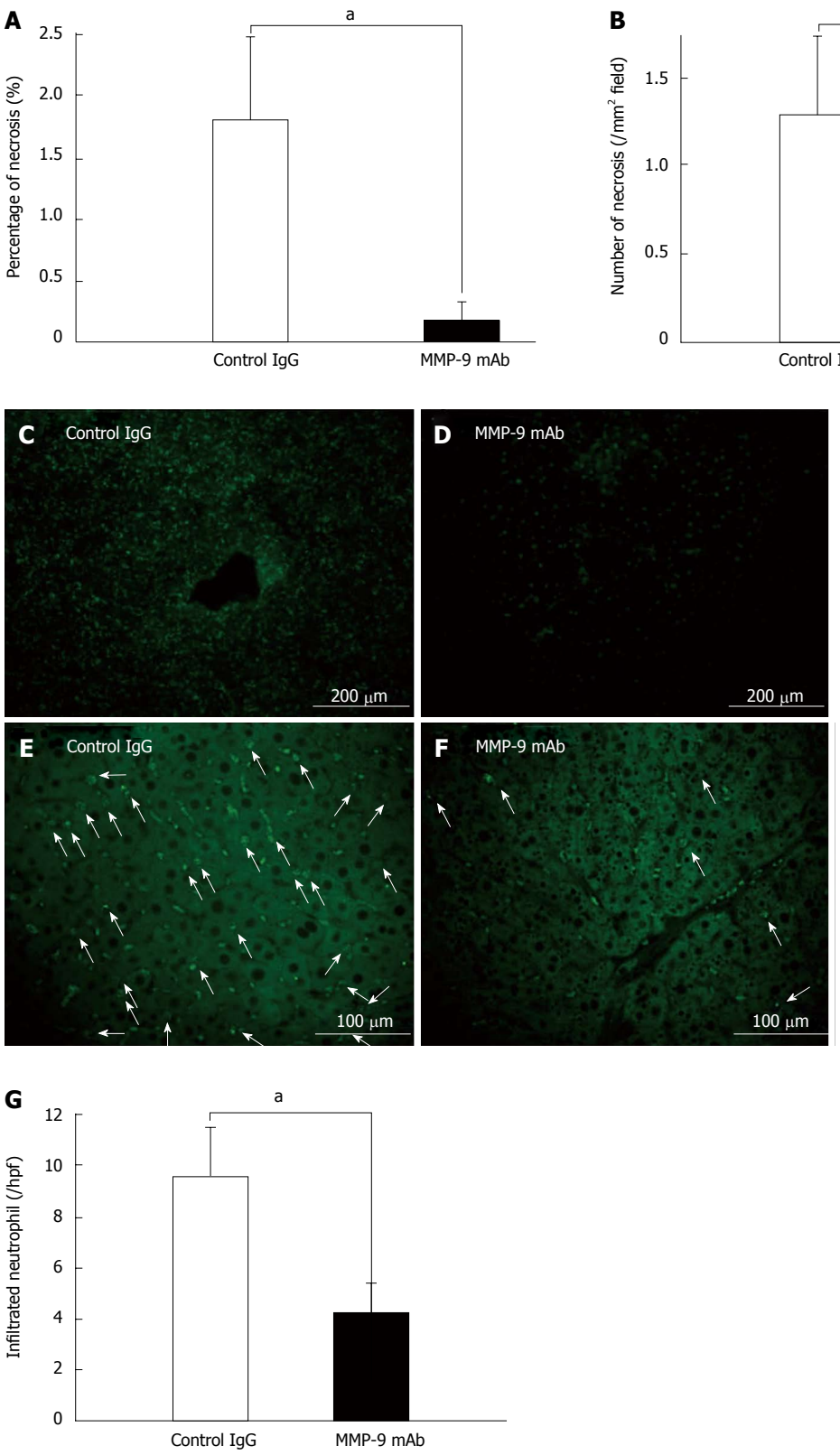

Figure 8 Effect of matrix metalloproteinase-9 monoclonal antibody on 80%-partial hepatectomy. A, B: Treatment with matrix metalloproteinase-9 monoclonal antibody (MMP-9 mAb) in mice with 80%-partial hepatectomy (PH) resulted in a significant reduction in the size (percent area) and number of hemorrhagic and necrotic foci 6 h after 80%-PH compared with treatment using control IgG ($^aP < 0.05$); C, D: Suppression of MMP-9 activity as determined by *in situ* fluorescence gelatin zymography compared with control IgG-treated mice; E, F: There was significantly less myeloperoxidase staining (arrows) in remnant liver sections in mice treated with MMP-9 mAb compared with that in control IgG-treated mice; G: A histogram shows the number of accumulated neutrophils in the remnant liver 6 h after 80%-PH. Significantly fewer neutrophils were observed in MMP-9 mAb-treated mice than in control IgG-treated animals ($^aP < 0.05$).

Although MMP-9 can cause liver injury in IHR after extended liver resection or SFSGs after SOLT, MMP-9 is also essential for liver regeneration[50]. It has been shown that MMP-9 releases hepatic growth factor from the hepatic extracellular matrix during the priming phase of liver regeneration[51,52]. Together with its physiological tissue inhibitor, TIMP-1, MMP-9 participates in regulating the hepatocellular cell cycle and proliferation[51]. MMP-9 is temporally correlated to angiogenesis and regeneration in the liver. Therefore, prolonged inhibition of MMP-9 might be clinically undesirable. Recent studies have suggested that inhibition of MMP-9 to ameliorate the early formation of parenchymal hemorrhage and necrosis should probably be started within and be limited to the first 2 d after extended liver resection or SOLT with SFSGs[50,52]. However, the use of MMP-9 inhibition in the clinical management of IHR or SFSGs requires further investigation and understanding to optimize its therapeutic effectiveness.

After extended liver resection or SOLT with SFSGs, portal hyperperfusion is likely to occur. Although increased portal hypertension is important in liver regeneration, uncontrolled acute portal hypertension with portal hyperperfusion can be detrimental[21,25,53]. In SOLT with SFSGs, portal hypertension and hyperperfusion are known to contribute to SFSL failure[25,54,55]. In experimental models[25,53,56] and in clinical LT with SFSGs[8,9,25,54,55], decompression of portal hyperperfusion has been shown to significantly attenuate hepatic hemorrhage and necrosis[57,58]. Evidence suggests that surgical and anatomical reduction of portal hyperperfusion is important in the management of IHR and SFSGs.

The molecular mechanisms by which portal hyperperfusion affects the outcome of IHR after extended hepatectomy and SFSGs after SOLT are poorly understood. Shear stress has been shown to induce nitric oxide production in microvascular endothelial cells[59], which might upregulate MMP-9 synthesis and production[60]. Reduction of portal hyperperfusion could thus indirectly mitigate the effect of MMP-9 production. However, it is not known how portal hyperperfusion relates to MMP-9 with respect to sinusoidal injury leading to parenchymal hemorrhage and necrosis. Other potential important mediators in the injury of IHR or SFSGs include oxidative stress[61] and tumor necrosis factor[62]. Understanding the role of MMP-9 and other signaling pathways will increase our ability to develop therapeutic options to heal and prevent liver injury associated with IHR and SFSGs.

Neutrophils have been consistently implicated in ischemia-reperfusion injury[19,35]. Neutrophils might produce and release MMPs that contribute to initiating sinusoidal injury[14]. Conversely, hepatic MMP-9 might be derived from hepatic stellate cells or sinusoidal cells, including endothelial cells and Kupffer cells[29]. Hepatic-derived MMP-9 might then attract neutrophils[30] into the liver after an injury. Neutrophils could then initiate and/or propagate sinusoidal hemorrhage and progressive necrosis[35]. Therefore, targeting neutrophils for treatment could be important in some diseases and conditions.

The limitations of this study include the following. Although MMP-9 is implicated in the formation of parenchymal hemorrhage and necrosis in IHR after 80%-PH, our study did not address how and where MMP-9 acts on the sinusoidal elements. The cascade of nitric oxide synthetase, which is important in upregulating MMP-9, was not examined[59,60]. Even though we did not observe constitutive MMP-9 in association with parenchymal hemorrhage and necrosis, we could not rule out the possibility that hepatic cells, including sinusoidal endothelial cells, Kupffer cells, and hepatic stellate cells[30], produced MMP-9 at a minute level that was adequate to initiate liver injury with subsequent recruitment of circulating neutrophils. The potential role of T cells was not evaluated[63]. The possibility of myeloperoxidase-associated oxidative inactivation of TIMP-1, thus activating MMP-9, was not considered in this study[64]. We consider that the effects of upregulation of MMP-9 on sinusoidal and endothelial cells should be investigated in detail.

Initially, we hypothesized that MMP-9 plays an important role in parenchymal hemorrhage and necrosis in the small remnant liver. We found significantly less hemorrhagic and necrotic lesions in MMP-9(-/-) remnant livers than in WT and TIMP-1(-/-) remnant livers. Similar results were observed with MMP-9 monoclonal antibody or the synthetic inhibitor GM6001. In conclusion, we demonstrated that MMP-9 plays an important role in the development of hepatic hemorrhage and necrosis in the small liver remnant 6 h after extensive partial hepatectomy. Moreover, we showed that infiltrating neutrophils are critical to the process of liver injury. Targeting MMP-9 could provide a clinically useful tool to optimize the function of a small liver volume, i.e., IHR after extended hepatectomy or SFSGs after SOLT.

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COMMENTS

Background

In the field of hepatobiliary surgery, liver resection is considered the standard treatment for primary liver tumors and colorectal liver metastases. Major liver resections have been associated with an increased morbidity and mortality compared with more limited resections. In particular, the outcomes are worsened when a chronic underlying liver disease is present. Recent studies have shown that the volume of the remnant liver is correlated with perioperative morbidity and mortality, and an insufficient hepatic remnant (IHR) after extended hepatectomy is a critical issue.

Research frontiers

Matrix metalloproteinases (MMPs) comprise a family of zinc-dependent neutral proteases that can degrade the extracellular matrix and basement membrane.

MMPs play significant roles in cellular regulation, cell-cell communication, and tumor progression. MMP-2 and MMP-9 have been implicated in liver injury and remodeling. Knocking out MMP-9 and other MMPs attenuates liver injury associated with interferon treatments. MMP-9 contributes to the pathogenesis of experimental acute liver failure. Specifically, MMP-9 has been implicated in sinusoidal breakdown leading to extravasation of circulating cells and hemorrhage. MMP-9 plays a pivotal role in ischemia-reperfusion injury. When MMP-9 is blocked with a synthetic inhibitor or by MMP-9 gene deletion, it successfully reverses ischemia-reperfusion injury. Collectively, these data show that MMP-9 is involved in sinusoidal injury in liver failure. However, the role of MMP-9 in the pathogenesis of failure in IHR is not well understood.

Innovations and breakthroughs

Initially, the authors hypothesized that MMP-9 plays an important role in parenchymal hemorrhage and necrosis in the small remnant liver. In this study, the authors found significantly less hemorrhagic and necrotic lesions in MMP-9(-/-) remnant livers than in wild-type and tissue inhibitors of metalloproteinases (TIMP)-1(-/-) remnant livers. Similar results were observed with a MMP-9 monoclonal antibody or synthetic inhibitor GM6001.

Applications

The authors demonstrated that MMP-9 plays an important role in the development of hepatic hemorrhage and necrosis in the small remnant liver 6 h after extensive partial hepatectomy. Moreover, the authors showed that infiltrating neutrophils are critical to the process of liver injury.

Terminology

Targeting MMP-9 could provide a clinically useful tool to optimize the function of a small liver volume, i.e., IHR after extended hepatectomy.

Peer review

Using MMP-9(-/-), wild type, and TIMP-1(-/-) mice, the authors demonstrated that in IHR at 6 h postoperatively MMP-9 is upregulated in the infiltrating neutrophils associated with the foci of hemorrhage and necrosis. Blocking MMP-9 expression by using a synthetic inhibitor, specific monoclonal antibodies, or gene deletion significantly ameliorated the formation of parenchymal hemorrhage and necrosis. The authors finally proposed that MMP is tightly associated with liver parenchymal hemorrhage and necrosis for insufficient liver remnant, which always leads a lethal outcome clinically.

REFERENCES

1 **Simmonds PC**, Primrose JN, Colquitt JL, Garden OJ, Poston GJ, Rees M. Surgical resection of hepatic metastases from colorectal cancer: a systematic review of published studies. *Br J Cancer* 2006; **94**: 982-999

2 **Jaeck D**, Bachellier P, Oussoultzoglou E, Weber JC, Wolf P. Surgical resection of hepatocellular carcinoma. Post-operative outcome and long-term results in Europe: an overview. *Liver Transpl* 2004; **10**: S58-S63

3 **Nagino M**, Kamiya J, Nishio H, Ebata T, Arai T, Nimura Y. Two hundred forty consecutive portal vein embolizations before extended hepatectomy for biliary cancer: surgical outcome and long-term follow-up. *Ann Surg* 2006; **243**: 364-372

4 **Belghiti J**, Hiramatsu K, Benoist S, Massault P, Sauvanet A, Farges O. Seven hundred forty-seven hepatectomies in the 1990s: an update to evaluate the actual risk of liver resection. *J Am Coll Surg* 2000; **191**: 38-46

5 **Shoup M**, Gonen M, D'Angelica M, Jarnagin WR, DeMatteo RP, Schwartz LH, Tuorto S, Blumgart LH, Fong Y. Volumetric analysis predicts hepatic dysfunction in patients undergoing major liver resection. *J Gastrointest Surg* 2003; **7**: 325-330

6 **Ferrero A**, Viganò L, Polastri R, Muratore A, Eminefendic H, Regge D, Capussotti L. Postoperative liver dysfunction and future remnant liver: where is the limit? Results of a prospective study. *World J Surg* 2007; **31**: 1643-1651

7 **Seyama Y**, Makuuchi M. Current surgical treatment for bile duct cancer. *World J Gastroenterol* 2007; **13**: 1505-1515

8 **Hori T**, Uemoto S, Gardner LB, Sibulesky L, Ogura Y, Nguyen JH. Left-sided grafts for living-donor liver transplantation and split grafts for deceased-donor liver transplantation: their impact on long-term survival. *Clin Res Hepatol Gastroenterol* 2012; **36**: 47-52

9 **Ogura Y**, Hori T, El Moghazy WM, Yoshizawa A, Oike F, Mori A, Kaido T, Takada Y, Uemoto S. Portal pressure < 15 mm Hg is a key for successful adult living donor liver transplantation utilizing smaller grafts than before. *Liver Transpl* 2010; **16**: 718-728

10 **van den Broek MA**, Olde Damink SW, Dejong CH, Lang H, Malagó M, Jalan R, Saner FH. Liver failure after partial hepatic resection: definition, pathophysiology, risk factors and treatment. *Liver Int* 2008; **28**: 767-780

11 **Emond JC**, Renz JF, Ferrell LD, Rosenthal P, Lim RC, Roberts JP, Lake JR, Ascher NL. Functional analysis of grafts from living donors. Implications for the treatment of older recipients. *Ann Surg* 1996; **224**: 544-552; discussion 552-554

12 **Dahm F**, Georgiev P, Clavien PA. Small-for-size syndrome after partial liver transplantation: definition, mechanisms of disease and clinical implications. *Am J Transplant* 2005; **5**: 2605-2610

13 **Rudich N**, Zamir G, Pappo O, Shlomai Z, Faroja M, Weiss ID, Wald H, Galun E, Peled A, Wald O. Focal liver necrosis appears early after partial hepatectomy and is dependent on T cells and antigen delivery from the gut. *Liver Int* 2009; **29**: 1273-1284

14 **Ito Y**, Abril ER, Bethea NW, McCuskey MK, Cover C, Jaeschke H, McCuskey RS. Mechanisms and pathophysiological implications of sinusoidal endothelial cell gap formation following treatment with galactosamine/endotoxin in mice. *Am J Physiol Gastrointest Liver Physiol* 2006; **291**: G211-G218

15 **Toth B**, Wilson RB. Early vascular injury induced by 1,2-dimethylhydrazine dihydrochloride. II. Gross and electron microscopic findings. *Arch Pathol* 1972; **93**: 427-434

16 **Chosay JG**, Essani NA, Dunn CJ, Jaeschke H. Neutrophil margination and extravasation in sinusoids and venules of liver during endotoxin-induced injury. *Am J Physiol* 1997; **272**: G1195-G1200

17 **Takenaka K**, Sakaida I, Yasunaga M, Okita K. Ultrastructural study of development of hepatic necrosis induced by TNF-alpha and D-galactosamine. *Dig Dis Sci* 1998; **43**: 887-892

18 **Wack KE**, Ross MA, Zegarra V, Sysko LR, Watkins SC, Stolz DB. Sinusoidal ultrastructure evaluated during the revascularization of regenerating rat liver. *Hepatology* 2001; **33**: 363-378

19 **Tsuchihashi S**, Ke B, Kaldas F, Flynn E, Busuttil RW, Briscoe DM, Kupiec-Weglinski JW. Vascular endothelial growth factor antagonist modulates leukocyte trafficking and protects mouse livers against ischemia/reperfusion injury. *Am J Pathol* 2006; **168**: 695-705

20 **Yachida S**, Kokudo Y, Wakabayashi H, Maeba T, Kaneda K, Maeta H. Morphological and functional alterations to sinusoidal endothelial cells in the early phase of endotoxin-induced liver failure after partial hepatectomy in rats. *Virchows Arch* 1998; **433**: 173-181

21 **Kuroda H**, Kawarada Y, Das BC, Iwata M, Isaji S. Ultrastructural study of the remnant liver after extensive hepatectomy in dogs; especially morphological alterations of sinusoidal endothelial cells. *Hepatogastroenterology* 2000; **47**: 450-454

22 **Man K**, Lo CM, Ng IO, Wong YC, Qin LF, Fan ST, Wong J. Liver transplantation in rats using small-for-size grafts: a study of hemodynamic and morphological changes. *Arch Surg* 2001; **136**: 280-285

23 **Tian Y**, Rüdiger HA, Jochum W, Clavien PA. Comparison of arterialized and nonarterialized orthotopic liver transplantation in mice: prowess or relevant model? *Transplantation* 2002; **74**: 1242-1246

24 **Yao A**, Li X, Pu L, Zhong J, Liu X, Yu Y, Zhang F, Kong L, Sun B, Wang X. Impaired hepatic regeneration by ischemic preconditioning in a rat model of small-for-size liver transplantation. *Transpl Immunol* 2007; **18**: 37-43

25 **Wang H**, Ohkohchi N, Enomoto Y, Usuda M, Miyagi S, Masuoka H, Sekiguchi S, Kawagishi N, Fujimori K, Sato A, Satomi S. Effect of portocaval shunt on residual extreme small

liver after extended hepatectomy in porcine. *World J Surg* 2006; **30**: 2014-2022; discussion 2023-2024
26 **Sternlicht MD**, Werb Z. How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol* 2001; **17**: 463-516
27 **Cauwe B**, Van den Steen PE, Opdenakker G. The biochemical, biological, and pathological kaleidoscope of cell surface substrates processed by matrix metalloproteinases. *Crit Rev Biochem Mol Biol* 2007; **42**: 113-185
28 **Wielockx B**, Lannoy K, Shapiro SD, Itoh T, Itohara S, Vandekerckhove J, Libert C. Inhibition of matrix metalloproteinases blocks lethal hepatitis and apoptosis induced by tumor necrosis factor and allows safe antitumor therapy. *Nat Med* 2001; **7**: 1202-1208
29 **Yan C**, Zhou L, Han YP. Contribution of hepatic stellate cells and matrix metalloproteinase 9 in acute liver failure. *Liver Int* 2008; **28**: 959-971
30 **Hanumegowda UM**, Copple BL, Shibuya M, Malle E, Ganey PE, Roth RA. Basement membrane and matrix metalloproteinases in monocrotaline-induced liver injury. *Toxicol Sci* 2003; **76**: 237-246
31 **Padrissa-Altés S**, Zaouali MA, Franco-Gou R, Bartrons R, Boillot O, Rimola A, Arroyo V, Rodés J, Peralta C, Roselló-Catafau J. Matrix metalloproteinase 2 in reduced-size liver transplantation: beyond the matrix. *Am J Transplant* 2010; **10**: 1167-1177
32 **Ma ZY**, Qian JM, Rui XH, Wang FR, Wang QW, Cui YY, Peng ZH. Inhibition of matrix metalloproteinase-9 attenuates acute small-for-size liver graft injury in rats. *Am J Transplant* 2010; **10**: 784-795
33 **Coito AJ.** Leukocyte transmigration across endothelial and extracellular matrix protein barriers in liver ischemia/reperfusion injury. *Curr Opin Organ Transplant* 2010; Epub ahead of print
34 **Tarrats N**, Moles A, Morales A, García-Ruiz C, Fernández-Checa JC, Marí M. Critical role of tumor necrosis factor receptor 1, but not 2, in hepatic stellate cell proliferation, extracellular matrix remodeling, and liver fibrogenesis. *Hepatology* 2011; **54**: 319-327
35 **Defamie V**, Laurens M, Patrono D, Devel L, Brault A, Saint-Paul MC, Yiotakis A, Barbry P, Gugenheim J, Crenesse D, Dive V, Huet PM, Mari B. Matrix metalloproteinase inhibition protects rat livers from prolonged cold ischemia-warm reperfusion injury. *Hepatology* 2008; **47**: 177-185
36 **Fujimoto M**, Takagi Y, Aoki T, Hayase M, Marumo T, Gomi M, Nishimura M, Kataoka H, Hashimoto N, Nozaki K. Tissue inhibitor of metalloproteinases protect blood-brain barrier disruption in focal cerebral ischemia. *J Cereb Blood Flow Metab* 2008; **28**: 1674-1685
37 **Tejima E**, Guo S, Murata Y, Arai K, Lok J, van Leyen K, Rosell A, Wang X, Lo EH. Neuroprotective effects of overexpressing tissue inhibitor of metalloproteinase TIMP-1. *J Neurotrauma* 2009; **26**: 1935-1941
38 **Hori T,** Ohashi N, Chen F, Baine AMT, Gardner LB, Hata T, Uemoto S, Nguyen JH. Simple and reproducible hepatectomy in the mouse using the clip technique. *World J Gastroenterol* 2012; In press
39 **Makino H**, Togo S, Kubota T, Morioka D, Morita T, Kobayashi T, Tanaka K, Shimizu T, Matsuo K, Nagashima Y, Shimada H. A good model of hepatic failure after excessive hepatectomy in mice. *J Surg Res* 2005; **127**: 171-176
40 **Nguyen JH**, Yamamoto S, Steers J, Sevlever D, Lin W, Shimojima N, Castanedes-Casey M, Genco P, Golde T, Richelson E, Dickson D, McKinney M, Eckman CB. Matrix metalloproteinase-9 contributes to brain extravasation and edema in fulminant hepatic failure mice. *J Hepatol* 2006; **44**: 1105-1114
41 **Hori T,** Uemoto S, Zhao X, Chen F, Baine AMT, Gardner LB, Ohashi N, Conkle F, Castanedes-Casey M, Phillips VR, Rousseau LG, Murray M, Kamo N, Nguyen JH. Surgical guide including innovative techniques for orthotopic liver transplantation in the rat: Key techniques and pitfalls in whole and split liver grafts. *Annals Gastroenterol* 2010; **23**: 270-295
42 **Matkowskyj KA**, Marrero JA, Carroll RE, Danilkovich AV, Green RM, Benya RV. Azoxymethane-induced fulminant hepatic failure in C57BL/6J mice: characterization of a new animal model. *Am J Physiol* 1999; **277**: G455-G462
43 **Bélanger M**, Côté J, Butterworth RF. Neurobiological characterization of an azoxymethane mouse model of acute liver failure. *Neurochem Int* 2006; **48**: 434-440
44 **Panis Y**, McMullan DM, Emond JC. Progressive necrosis after hepatectomy and the pathophysiology of liver failure after massive resection. *Surgery* 1997; **121**: 142-149
45 **Mitchell C**, Willenbring H. A reproducible and well-tolerated method for 2/3 partial hepatectomy in mice. *Nat Protoc* 2008; **3**: 1167-1170
46 **Jin X**, Zhang Z, Beer-Stolz D, Zimmers TA, Koniaris LG. Interleukin-6 inhibits oxidative injury and necrosis after extreme liver resection. *Hepatology* 2007; **46**: 802-812
47 **Shen ZY**, Zhu ZJ, Deng YL, Zheng H, Pan C, Zhang YM, Shi R, Jiang WT, Zhang JJ. Liver retransplantation: report of 80 cases and review of literature. *Hepatobiliary Pancreat Dis Int* 2006; **5**: 180-184
48 **Shen XD**, Ke B, Zhai Y, Gao F, Anselmo D, Lassman CR, Busuttil RW, Kupiec-Weglinski JW. Stat4 and Stat6 signaling in hepatic ischemia/reperfusion injury in mice: HO-1 dependence of Stat4 disruption-mediated cytoprotection. *Hepatology* 2003; **37**: 296-303
49 **Cursio R**, Mari B, Louis K, Rostagno P, Saint-Paul MC, Giudicelli J, Bottero V, Anglard P, Yiotakis A, Dive V, Gugenheim J, Auberger P. Rat liver injury after normothermic ischemia is prevented by a phosphinic matrix metalloproteinase inhibitor. *FASEB J* 2002; **16**: 93-95
50 **Alwayn IP**, Verbesey JE, Kim S, Roy R, Arsenault DA, Greene AK, Novak K, Laforme A, Lee S, Moses MA, Puder M. A critical role for matrix metalloproteinases in liver regeneration. *J Surg Res* 2008; **145**: 192-198
51 **Mohammed FF**, Pennington CJ, Kassiri Z, Rubin JS, Soloway PD, Ruther U, Edwards DR, Khokha R. Metalloproteinase inhibitor TIMP-1 affects hepatocyte cell cycle via HGF activation in murine liver regeneration. *Hepatology* 2005; **41**: 857-867
52 **Olle EW**, Ren X, McClintock SD, Warner RL, Deogracias MP, Johnson KJ, Colletti LM. Matrix metalloproteinase-9 is an important factor in hepatic regeneration after partial hepatectomy in mice. *Hepatology* 2006; **44**: 540-549
53 **Ueno S**, Kobayashi Y, Kurita K, Tanabe G, Aikou T. Effect of prior portosystemic shunt on early hepatic hemodynamics and sinusoids following 84% hepatectomy in dogs. *Res Exp Med* (*Berl*) 1995; **195**: 1-8
54 **Kiuchi T**, Kasahara M, Uryuhara K, Inomata Y, Uemoto S, Asonuma K, Egawa H, Fujita S, Hayashi M, Tanaka K. Impact of graft size mismatching on graft prognosis in liver transplantation from living donors. *Transplantation* 1999; **67**: 321-327
55 **Demetris AJ**, Kelly DM, Eghtesad B, Fontes P, Wallis Marsh J, Tom K, Tan HP, Shaw-Stiffel T, Boig L, Novelli P, Planinsic R, Fung JJ, Marcos A. Pathophysiologic observations and histopathologic recognition of the portal hyperperfusion or small-for-size syndrome. *Am J Surg Pathol* 2006; **30**: 986-993
56 **Ku Y**, Fukumoto T, Nishida T, Tominaga M, Maeda I, Kitagawa T, Takao S, Shiotani M, Tseng A, Kuroda Y. Evidence that portal vein decompression improves survival of canine quarter orthotopic liver transplantation. *Transplantation* 1995; **59**: 1388-1392
57 **Troisi R**, Ricciardi S, Smeets P, Petrovic M, Van Maele G, Colle I, Van Vlierberghe H, de Hemptinne B. Effects of hemiportocaval shunts for inflow modulation on the outcome of small-for-size grafts in living donor liver transplantation. *Am J Transplant* 2005; **5**: 1397-1404
58 **Umeda Y**, Yagi T, Sadamori H, Matsukawa H, Matsuda H, Shinoura S, Mizuno K, Yoshida R, Iwamoto T, Satoh D,

Tanaka N. Effects of prophylactic splenic artery modulation on portal overperfusion and liver regeneration in small-for-size graft. *Transplantation* 2008; **86**: 673-680

59 **Dumont O**, Loufrani L, Henrion D. Key role of the NO-pathway and matrix metalloprotease-9 in high blood flow-induced remodeling of rat resistance arteries. *Arterioscler Thromb Vasc Biol* 2007; **27**: 317-324

60 **Misra S**, Fu AA, Anderson JL, Sethi S, Glockner JF, McKusick MA, Bjarnason H, Woodrum DA, Mukhopadhyay D. The rat femoral arteriovenous fistula model: increased expression of matrix metalloproteinase-2 and -9 at the venous stenosis. *J Vasc Interv Radiol* 2008; **19**: 587-594

61 **Moon KH**, Hood BL, Mukhopadhyay P, Rajesh M, Abdelmegeed MA, Kwon YI, Conrads TP, Veenstra TD, Song BJ, Pacher P. Oxidative inactivation of key mitochondrial proteins leads to dysfunction and injury in hepatic ischemia reperfusion. *Gastroenterology* 2008; **135**: 1344-1357

62 **Tian Y**, Jochum W, Georgiev P, Moritz W, Graf R, Clavien PA. Kupffer cell-dependent TNF-alpha signaling mediates injury in the arterialized small-for-size liver transplantation in the mouse. *Proc Natl Acad Sci USA* 2006; **103**: 4598-4603

63 **Khandoga A**, Hanschen M, Kessler JS, Krombach F. CD4+ T cells contribute to postischemic liver injury in mice by interacting with sinusoidal endothelium and platelets. *Hepatology* 2006; **43**: 306-315

64 **Wang Y**, Rosen H, Madtes DK, Shao B, Martin TR, Heinecke JW, Fu X. Myeloperoxidase inactivates TIMP-1 by oxidizing its N-terminal cysteine residue: an oxidative mechanism for regulating proteolysis during inflammation. *J Biol Chem* 2007; **282**: 31826-31834

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ORIGINAL ARTICLE

Anti-tumor effect of 5-aza-2'-deoxycytidine by inhibiting telomerase activity in hepatocellular carcinoma cells

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Abstract

AIM: To investigate the effect of the demethylating reagent 5-aza-2′-deoxycitidine (DAC) on telomerase activity in hepatocellular carcinoma (HCC) cell lines, SMMC-7721 and HepG2.

METHODS: The related gene expression in cell lines was examined by real-time reverse transcription-polymerase chain reaction and Western blotting analysis. The telomerase activity was examined by telomeric repeat amplification protocol-enzyme-linked immunosorbent assay and DNA methylation was determined by methylation-specific polymerase chain reaction.

RESULTS: The telomerase activity was significantly reduced in both cell lines treated with DAC, accompanied by downregulation of telomerase reverse transcriptase (hTERT). We also observed the effect of DAC on the methylation status of hTERT promoter and the expression of regulatory genes, such as c-myc, p15, p16, p21, E2F1, and WT1. The methylation status of hTERT promoter could be reversed in SMMC-7721 by DAC, but not in HepG2 cells. However, p16 expression could be reactivated by demethylation of its promoter, and c-Myc expression was repressed in both cell lines. Moreover, DAC could enhance the sensitivity to the chemotherapeutic agents, such as cisplatin, by induction of apoptosis of HCC cells.

CONCLUSION: The DAC exerts its anti-tumor effects in HCC cells by inhibiting the telomerase activity.

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Key words: 5-aza-2′-deoxycitidine; Telomerase; Hepatocellular carcinoma; DNA methylation

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INTRODUCTION

The demethylating reagent 5-aza-2'-deoxycitidine (DAC) inhibits DNA methyltransferases and reverses DNA me-

thylation[1]. It has been found that DAC can inhibit cancer cell growth, particularly the leukemia cells, and it has been applied for the treatment of myelodysplastic syndromes. A phase 1 study was finished using DAC as an antineoplastic drug for hematopoietic malignancies[2,3]. However, the mechanisms underlying its anticancer activity and other biological effects are not fully understood. It is believed to reactivate genes, including the tumor suppressor genes p16, E-cadherin, and hMLH1 in cancer cells. Reactivation of these genes is associated with cell cycle arrest and apoptosis, which leads to inhibition of tumor cell growth[4]. However, the mechanisms of telomerase activity and telomerase reverse transcriptase (hTERT) down-regulated by DAC remain unclear. Telomerase is an RNA-dependent DNA polymerase that synthesizes telomeric DNA sequences and almost universally provides the molecular basis for tumor cell proliferative capacity[5]. Telomerase reactivation is a critical step in cellular immortality and carcinogenesis[6,7]. The enzyme consists of three major components: hTERT, telomerase associated protein (TEP1), and telomerase RNA (TERC)[8]. Transcriptional regulation of hTERT is believed to be the major mechanism of telomerase regulation in human cells. Transient transfection experiments with hTERT promoter-luciferase reporters showed that the hTERT promoter is inactive in normal and transformed preimmortal cells but, like telomerase, is activated in immortal cells[9]. Expression of hTERT was observed at high levels in malignant tumors and cancer cell lines, but not in normal tissues or telomerase-negative cell lines, and a strong correlation has been found between hTERT expression and telomerase activity in a variety of tumors[10,11]. These findings suggest that expression of hTERT might be a critical event in carcinogenesis. Thus, the mechanisms of hTERT activation are essential for understanding the molecular basis of telomerase activation and carcinogenesis. The hTERT promoter contains binding sites for many transcription factors that could be involved in its regulation. The abundance of these potential transcription factor binding sites was subjected to various factors in different cellular contexts[12,13]. Several transcription factors are known to participate in hTERT gene expression, including positive regulators: c-Myc, Sp1, human papillomavirus 16 E6, and steroid hormones; and negative regulators: Mad1, p53, p15, p16, p21, E2F, pRB, WT1, interferon-α, tumor growth factor-β and myeloid cell-specific zinc finger protein[14,15]. Our previous studies revealed that methylation status of P21, P15, P16, WTI and E2F-1 was significantly associated with cancer tissues in hepatocellular carcinoma (HCC)[16]. In the present study, we observed the effect of DAC on telomerase activity and hTERT expression in HCC cell lines, and found that DAC down-regulated the telomerase activity and hTERT expression by p16 promoter demethylation.

MATERIALS AND METHODS

Cell lines and cell culture

Human hepatoma cell lines SMMC-7721 and HepG2 were maintained in Dulbecco's modified Eagle's medium (high glucose) (Gibco, Invitrogen, United States) and supplemented with 10% fetal bovine serum (GIBCO, Invitrogen, United States), 100 units/mL penicillin, and 100 mg/mL streptomycin in a humidified incubator under 95% air and 5% CO_2 at 37 ℃. Cells from exponentially growing cultures were used in all the experiments. This study was approved by the Institutional Ethics Committee of the Second Military Medical University, China.

DAC

Human hepatoma cell lines (SMMC-7721 and HepG2) were seeded at a density of 5×10^5 cells per well in 6-well tissue culture plates and were allowed to attach over a 24-h period. The demethylating reagent DAC (Merk, Calbiochem, United States) was added to a final concentration of 1 μmol/L, 2 μmol/L and 4 μmol/L and the cells grew for 1 d, 3 d and 5 d. At indicated time intervals, cells were harvested by trypsinization and washed with phosphate-buffered saline.

Assay of telomerase activity by telomeric repeat amplification protocol-enzyme-linked immunosorbent assay

Telomeric repeat amplification protocol-enzyme-linked immunosorbent assay (TRAP-ELISA) was performed using the telomerase kit Telo TAGGG Telomerase PCR ELISA PLUS (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Extracts from HEK293 cells were used as positive controls for the assay, and cell lysates heat-inactivated for 10 min at 85 ℃ were used as negative controls. Absorbance values were reported as the $A_{450\ nm}$ reading.

Real-time reverse transcription-polymerase chain reaction

RNA was extracted from cells using Trizol (Invitrogen, Carlsbad, CA, United States). cDNA was synthesized using 2 μg total RNA and a moloney murine leukemia virus-reverse transcriptase kit with random hexamer primers and an RNase inhibitor (Takara Biotechnology Co. Ltd., Dalian, China). Polymerase chain reaction (PCR) amplifications of the respective genes were carried out with 40 ng complementary DNA, 500 nmol/L forward and reverse primer, and iTaqSYBRGreen Supermix (Bio-Rad Laboratories, Hercules, CA) in a final volume of 20 μL. Primer sequences and annealing temperature are summarized in Table 1. Real-time RT-PCR was performed on a Mastercycler cycler (Eppendorf, Hamburg, Germany), and all experiments were performed twice. Amplification of the housekeeping gene β-actin was performed to standardize the amount of sample RNA. Relative quantification of gene expression was performed by the -ΔΔct method.

Methylation-specific polymerase chain reaction

The bisulfite modification of DNA was done as described previously[17]. DNA methylation was determined

Table 1 Primer sequences for reverse transcription-polymerase chain reaction and methylation-specific polymerase chain reaction analysis

Gene	Primer sequence (5′-3′)	Annealing (℃)	Product size (bp)
hTERT-F	CGGAAGAGTGTCTGGAGCAA	58	422
hTERT-R	GGATGAAGCGGAGTCTGGA		
c-myc-F	CTTCTCTCCGTCCTCGGATTCT	65	132
c-myc-R	GAAGGTGATCCAGACTCTGACCTT		
p15-F	GAATGCGCGAGGAGAACAAG	65	204
p15-R	CCATCATCATGACCTGGATCG		
p16-F	GCTGCCCACGCACCGAATA	57	179
p16-R	ACCACCAGCGTGTCCAGGAA		
p21-F	GCAGACCAGCATGACAGATTT	60	70
p21-R	GGATTAGGGCTTCCTCTTGGA		
WT1-F	GGCATCTGAGACCAGTGAGAA	62	120
WT1-R	GAGAGTCAGACTTGAAAGCAGT		
E2F1-F	AGCTGGACCACCTGATGAAT	60	95
E2F1-R	GTCCTGACACGTCACGTAGG		
β-actin-F	CCTGTACGCCAACACAGTGC	60	275
β-actin-R	ATACTCCTGCTTGCTGATCC		
hTERT-MF	AGTTTTGGTTTCGGTTATTTTCGC	58	122
hTERT-MR	AACGTAACCAACGACAACACC		
hTERT-UF	AGTTTTGGTTTTGGTTATTTTTGT	58	132
hTERT-UR	AACGTAACCAACGACAACACCT		
p16-MF	TTATTAGAGGGTGGGGCGGATCGC	56	150
p16-MR	GACCCCGAACCGCGACCGTAA		
p16-UF	TTATTAGAGGGTGGGGTGGATTGT	56	151
p16-UR	CAACCCCAAACCACAACCATAA		

by methylation-specific PCR (MSP). Forty ng of bisulfite-modified DNA was subjected to PCR amplification. The PCR reaction mixture contained 2.5 μL of 10 × PCR buffer, 100 pmol of each primer, 2 mmol/L of each dNTPs, and 1 U of Hotstart Taq DNA polymerase (Takara Biotechnology Co. Ltd., Dalian, China) at a final volume of 25 μL. The PCR was performed in a thermal cycler. Primer sequences and reaction conditions are summarized in Table 1. DNA methylated by SssI methylase (Sss DNA) was used as positive control for methylated alleles.

Western blotting analysis

HepG2 and SMMC-7721 cells were lysed in RIPA lysis buffer (Beyotime, China) with 1 mmol/L phenylmethanesulfonyl fluoride (PMSF). An equal amount of protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto the nitrocellulose membrane. After blocking with 5% nonfat milk, the membrane was probed with anti-hTERT (Santa Cruz, United States), developed with the BeyoECL Plus substrate system (Beyotime, China). Blots were stripped and re-probed with β-actin antibody (Santa Cruz, United States) to confirm equal protein loading.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide colorimetric assay and cell apoptosis analysis

The SMMC-7721 and HepG2 cells were seeded in 96-well plates and cultured with chemotherapeutic drugs and DAC for 3 d. The cells were examined by a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (5 mg/mL, Sigma) assay. The spectrophotometric absorbance was measured using a plate reader at 570 nm. The morphology of apoptosis was observed by 4',6-diamidino-2-phenylindole (DAPI) staining. The cells were analyzed using a Facial Action Coding System (FACS) Aria flow cytometer (Becton Dickinson, San Jose, CA).

Statistical analysis

All of the experiments were repeated at least three times. The data were expressed as means ± SD. Statistical analysis was performed using Student's *t* test (two tailed). $P < 0.05$ was considered statistically significant.

RESULTS

Telomerase activity in HCC cells with DAC

To investigate the effects of DAC on telomerase activity, SMMC-7721 and HepG2 were cultured with 1 μmol/L, 2 μmol/L and 4 μmol/L DAC. Telomerase activity was measured by TRAP-PCR-ELISA assay after 1 d, 3 d and 5 d of exposure to DAC. Inhibition of telomerase activity was observed in both cell lines in a dose-dependent manner, by maximal repression on day 3 at 4 μmol/L or day 5 at 2 μmol/L DAC (Figure 1). There was a 52.7% reduction of telomerase activity in SMMC-7721 cells treated with 4 μmol/L DAC for 3 d, and a 45.6% reduction of telomerase activity in HepG2 cells. The results revealed that the effect of DAC on telomerase activity varied in different cell lines.

Effect of DAC on telomerase reverse transcriptase expression in HCC cells

Since the expression of hTERT is closely associated with telomerase activity, we examined whether hTERT expression is suppressed in SMMC-7721 and HepG2 cells by DAC. The expression of hTERT mRNA in SMMC-7721 cells was decreased to 82% on day 1, 34% on day 3, and 26% on day 5 after DAC treatment (2 μmol/L) (Figure 2). A decline in hTERT mRNA was also detected in HepG2 cells treated with DAC. hTERT mRNA expression was maximally down-regulated by 4 μmol/L DAC. The complete down-regulation of hTERT mRNA became apparent on day 3 of treatment and maximal on day 5 in both cell lines. Furthermore, we treated SMMC-7721 and HepG2 cells with 1 μmol/L, 2 μmol/L, 4 μmol/L DAC respectively for 3 d and 2 μmol/L for 1 d, 3 d, 5 d, respectively, then detected the hTERT expression in protein level by Western blotting analysis (Figure 3). The hTERT protein in SMMC-7721 and HepG2 cells was also down-regulated by DAC in a dose- and time-dependent manner, with maximal repression at 4 μmol/L on day 5. The hTERT protein was notably suppressed in both HepG2 and SMMC-7721 cells after treated by 2 μmol/L DAC for 3 d; however, the effect was more significant in SMMC-7721 cells. These results were in accordance with hTERT mRNA expression. The results indicated that inhibition of telomerase activity in HCC cells treated with DAC may contribute to a striking decrease in hTERT

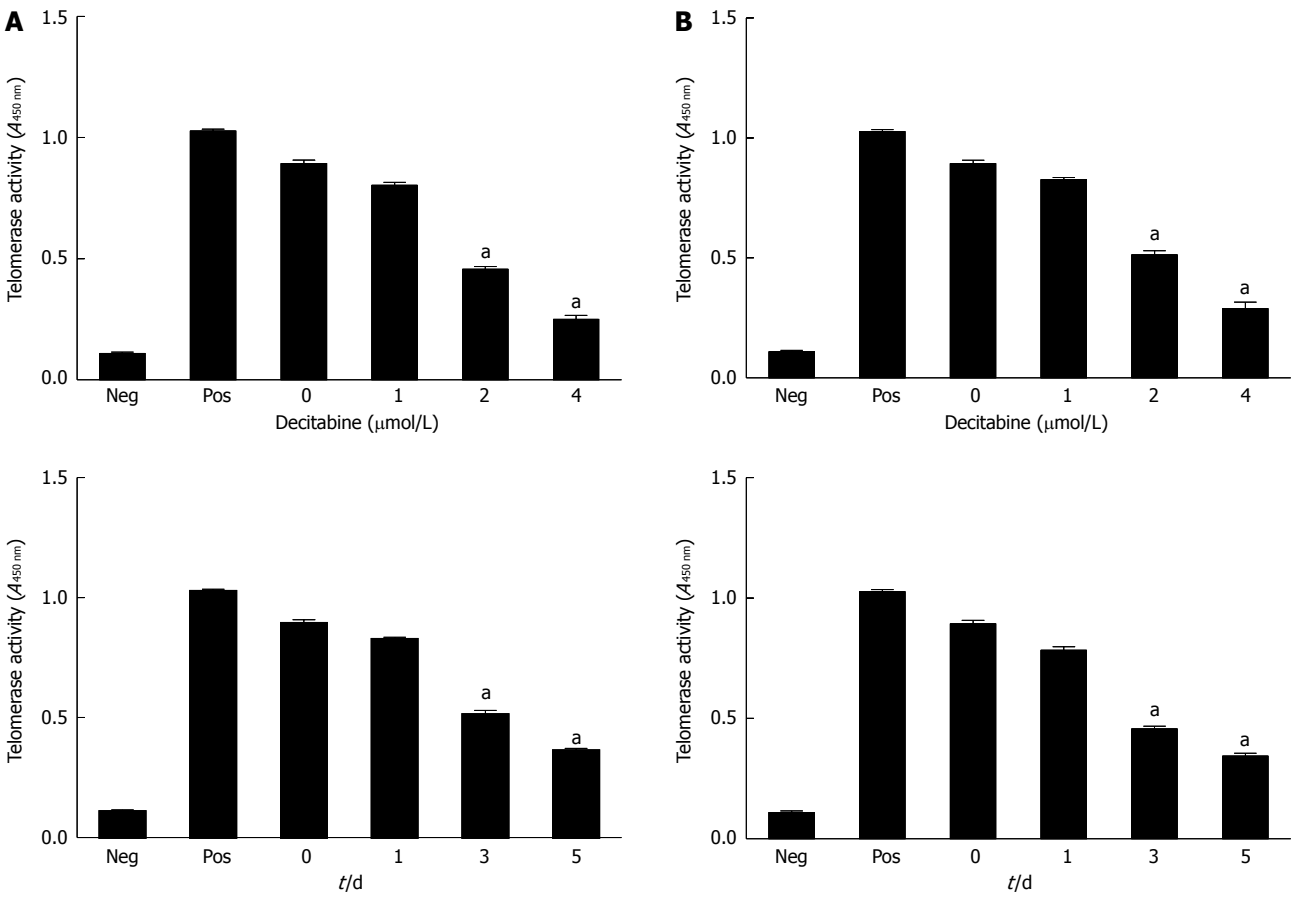

Figure 1 Effect of 5-aza-2'-deoxycitidine on telomerase activity in human hepatocellular carcinoma cell lines SMMC-7721 (A) and HepG2 (B). Cells were incubated with DAC (1 μmol/L, 2 μmol/L or 4 μmol/L). Cell pellets were collected and subjected to telomeric repeat amplification protocol-enzyme-linked immunosorbent assay. $^{a}P < 0.05$ (by unpaired Student's *t* test). Neg: Negative control; Pos: Positive control; DAC: 5-aza-2'-deoxycitidine. All studies are representative of at least three independent experiments.

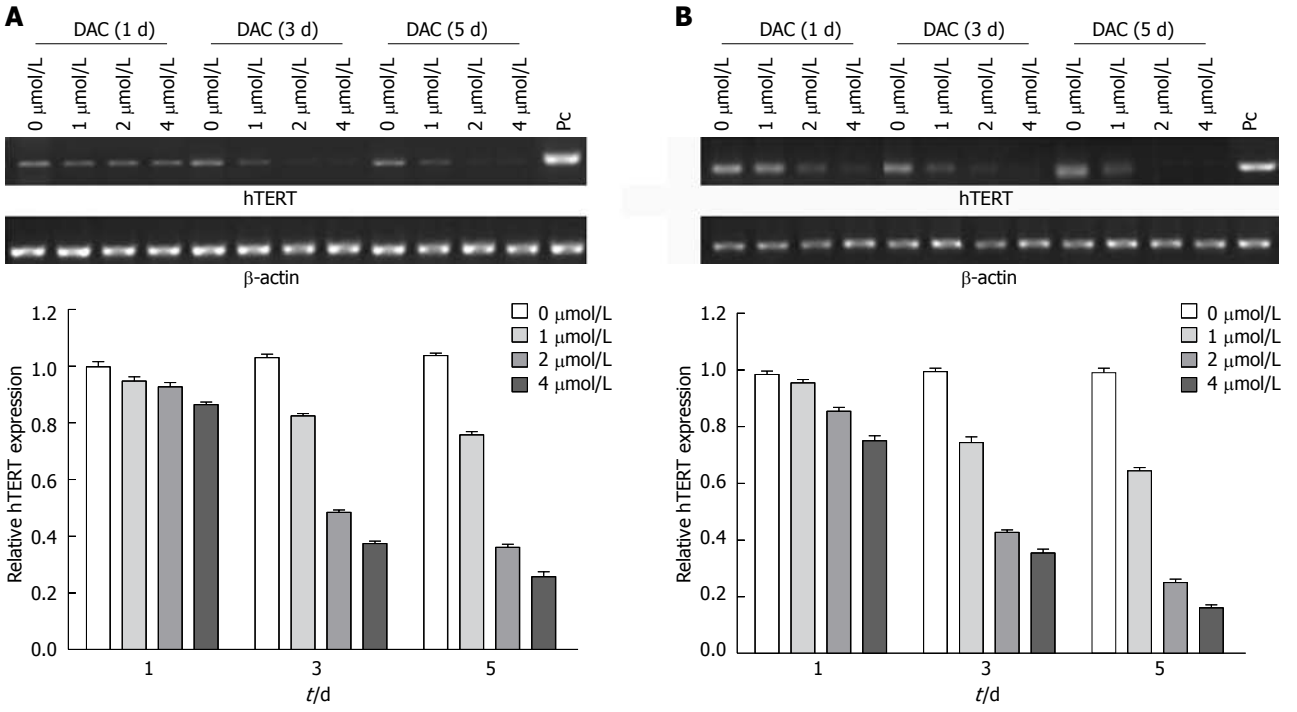

Figure 2 Effect of 5-aza-2'-deoxycitidine on telomerase reverse transcriptase mRNA in hepatocellular carcinoma cell lines SMMC-7721 (A) and HepG2 (B). Cells were incubated with DAC (1 μmol/L, 2 μmol/L or 4 μmol/L). Cell pellets were collected and subjected to real-time reverse transcription-polymerase chain reaction assay. PC: Positive control; DAC: 5-aza-2'-deoxycitidine; hTERT: Human telomerase reverse transcriptase.

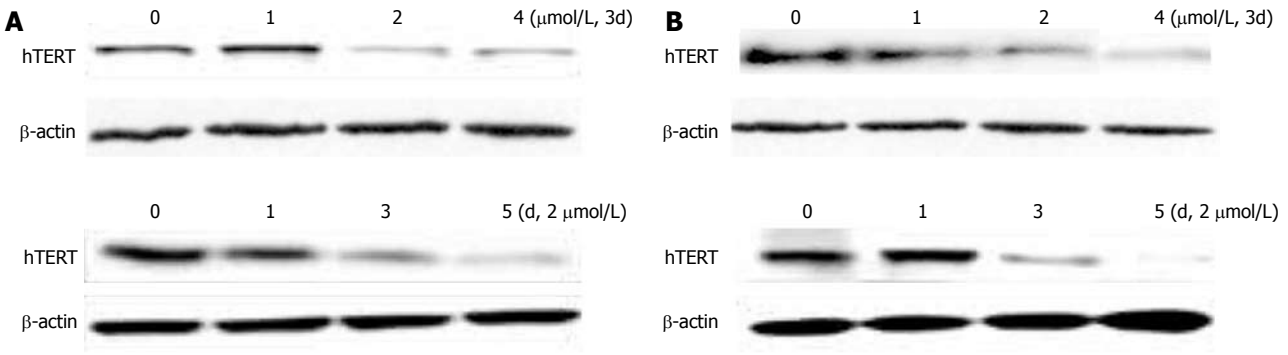

Figure 3 Expression of telomerase reverse transcriptase protein in hepatocellular carcinoma cell lines SMMC-7721 (A) and HepG2 (B) during exposure to 5-aza-2'-deoxycitidine (1 μmol/L, 2 μmol/L or 4 μmol/L). Total proteins were extracted, and Western blotting analysis was performed. The same blot was reprobed for β-actin as a loading control; hTERT: Human telomerase reverse transcriptase.

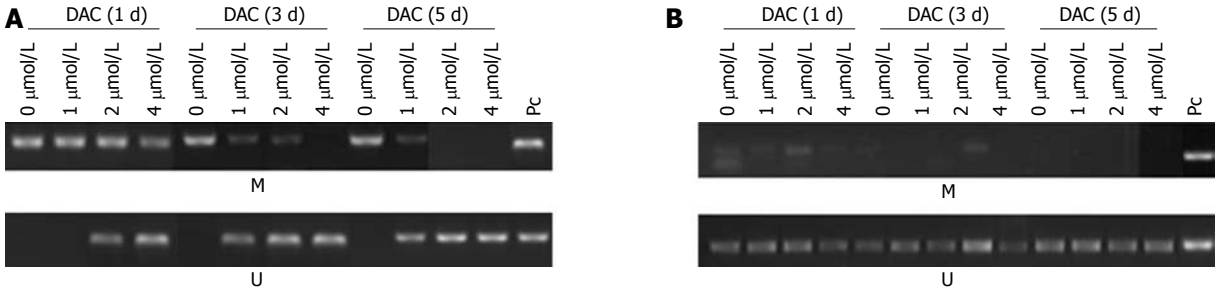

Figure 4 Methylation of telomerase reverse transcriptase promoter in 5-aza-2'-deoxycitidine-treated hepatocellular carcinoma cell lines SMMC-7721 (A) and HepG2 (B). M: Methylation; U: Unmethylation; PC: Positive control; DAC: 5-aza-2'-deoxycitidine.

mRNA and protein.

Methylation of telomerase reverse transcriptase promoter in HCC cells by DAC

Since promoter methylation may be involved in hTERT repression in HCC cells, we observed the effects of DAC on promoter methylation of hTERT gene using MSP[18]. According to MSP analysis, the hTERT promoter was found to be hypermethylated in SMMC-7721, but not in HepG2 cells (Figure 4). The demethylation of hTERT was found in SMMC-7721 cells treated with DAC in a dose- and time-dependent manner, and there was complete demethylation after treatment with 5 μmol/L DAC for 3 d or 2 μmol/L for 3 d (Figure 4A). However, DAC showed no effects on hTERT methylation in HepG2 cells (Figure 4B). These data suggested that the demethylation of hTERT promoter by DAC could not play an important role in down-regulation of hTERT expression.

Expression of critical regulatory genes of telomerase reverse transcriptase transcription by DAC

We focused on some regulatory genes of hTERT transcription, such as c-myc, p15, p16, p21, E2F-1, WT1. Using real-time PCR, we examined the mRNA expression of these genes during exposure to DAC at 2 μmol/L for 1 d, 3 d and 5 d. The data showed that c-myc had high expression while p16 had low expression (SMMC-7721) or lost expression (HepG2) in hepatoma cells, and the other genes showed different levels of expression (Figure 5). The down-regulation of c-myc and up-regulation of p16 mRNA expression were found in HCC cell lines treated with DAC. Western blotting analyses further revealed the significant levels of c-myc and p16 in both SMMC-7721 and HepG2 cells. These results suggested that c-myc and p16 could play an important role in down-regulating the hTERT expression by DAC.

Methylation of p16 promoter and reactivation of its expression by DAC

We reproduced the methylation pattern of p16 (Figure 6) using MSP. With DAC treatment, we detected increased demethylation of p16 promoter. The data suggested that promoter hypermethylation could causatively contribute to transcriptional silencing of p16, which up-regulated the hTERT transcription in HCC cells.

Human hepatoma cells sensitive to chemotherapeutic agents after DAC treatment

To determine whether DAC could effectively inhibit HCC cell growth, we treated SMMC-7721 and HepG2 cells with various doses of DAC for 72 h. We found that DAC inhibited the growth of cell lines in a dose-dependent manner (Figure 7). To determine whether the growth inhibition by DAC can be enhanced by chemotherapeutic agents, SMMC-7721 and HepG2 cells were treated with DAC in combination with cisplatin. Notably, while dramatic morphological changes were caused by combination of DAC with cisplatin, many cells revealed detachment and shrinkage. The growth inhibition was about 76% in

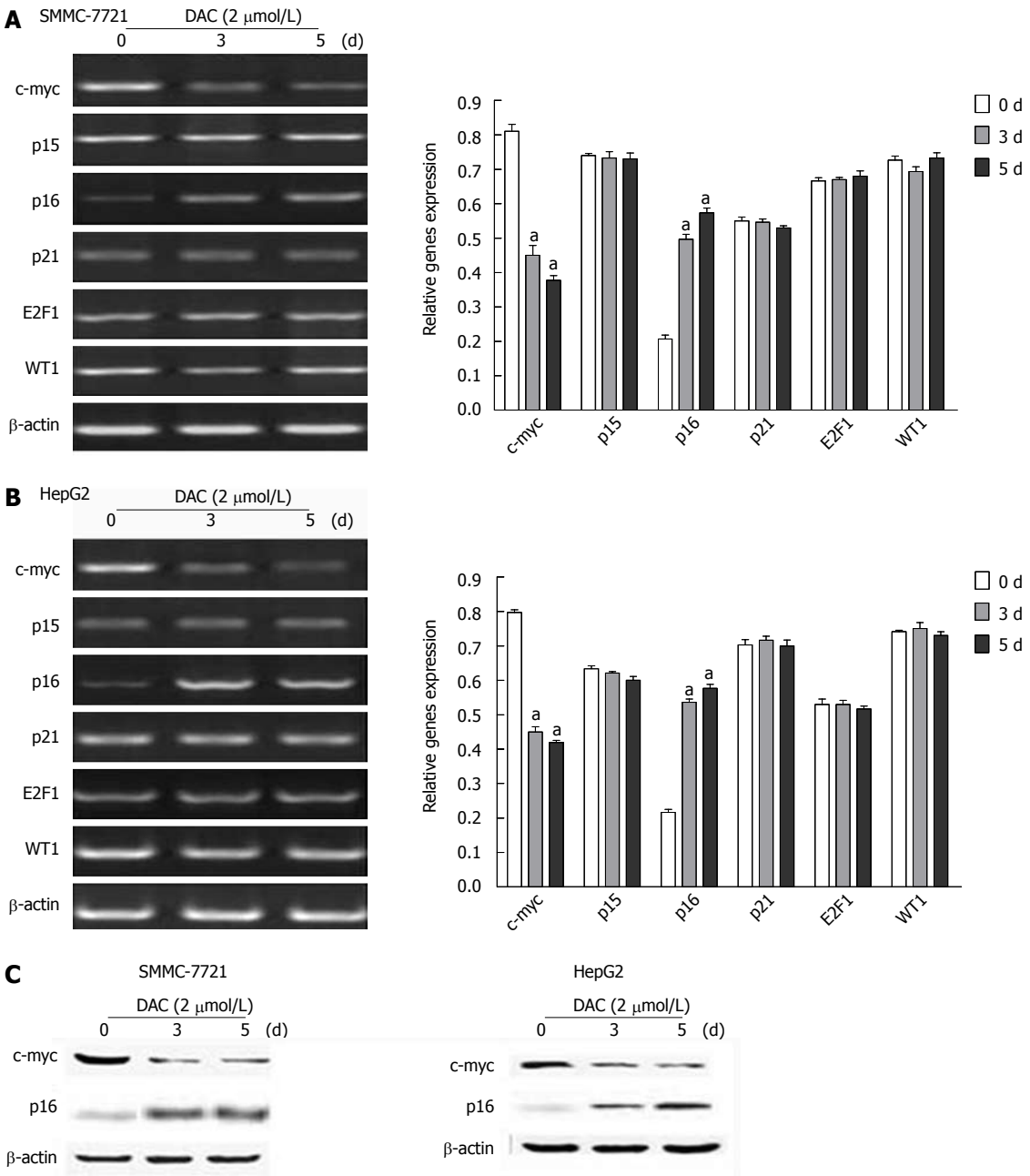

Figure 5 Effect of 5-aza-2'-deoxycitidine on expression of critical regulatory genes of telomerase reverse transcriptase transcription in hepatocellular carcinoma cell lines. A, B: Cells were incubated with 2 μmol/L DAC for 3-5 d, and subjected to real-time reverse transcription-polymerase chain reaction assay, $^{a}P < 0.05$ (by unpaired Student's *t* test); C: Western blotting analysis of c-myc and p16 in hepatocellular carcinoma cell lines by DAC. The same blot was reprobed for β-actin as a loading control. DAC: 5-aza-2'-deoxycitidine.

the cells treated with DAC in combination with cisplatin (20 μmol/L) as compared with 54% in the cells treated with cisplatin alone, or 48% treated with DAC alone. These data suggested that DAC could enhance the sensitivity of HCC cells to chemotherapeutic agents such as cisplatin. With the administration of cisplatin and DAC, more cells revealed nuclear condensation and fragmentation of apoptotic cell death. These results were confirmed by Annexin-V and propidium iodide staining and FACS analysis (Figure 8). The cell apoptosis was significantly enhanced by the combined administration of 2 μmol/L DAC and 20 μmol/L cisplatin. These results suggested that the enhanced effects of the combined treatment on cell death were attributed to the augmented induction of apoptosis.

DISCUSSION

The most widely used demethylating agent, DAC, was first characterized 30 years ago and it functions as a mechanism-dependent suicide inhibitor of DNA methyltransferases, with which genes silenced by hypermethylation can be reactivated[19]. It has been found that telomerase activity in cancer cells was inhibited by differ-

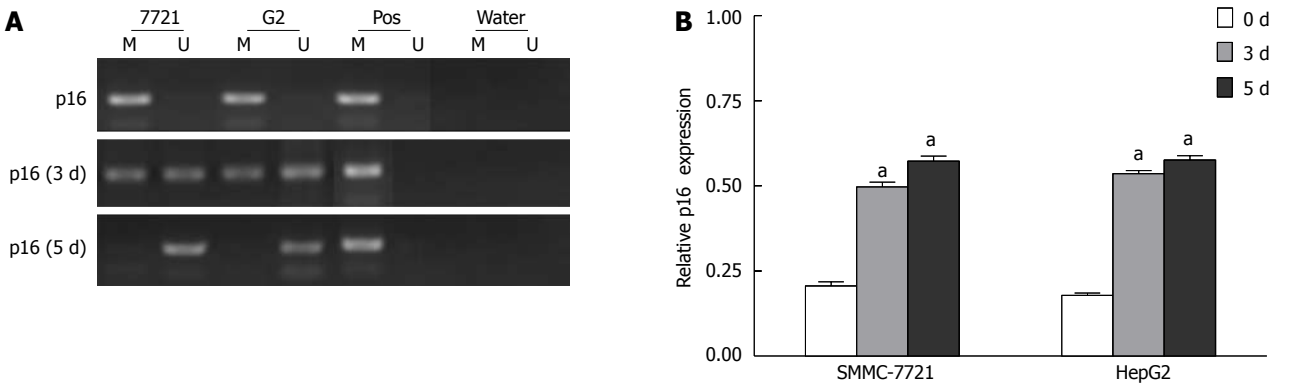

Figure 6 Demethylation of p16 promoter region and reactivation of p16 expression after 5-aza-2'-deoxycitidine treatment. Cells were incubated with 2 μmol/L DAC for 3-5 d and subjected to methylation-specific polymerase chain reaction (MSP) and real-time reverse transcription-polymerase chain reaction assay, respectively. M: Methylation; U: Unmethylation; Pos: Positive control. $^{a}P < 0.05$ (by unpaired Student's *t* test). DAC: 5-aza-2'-deoxycitidine.

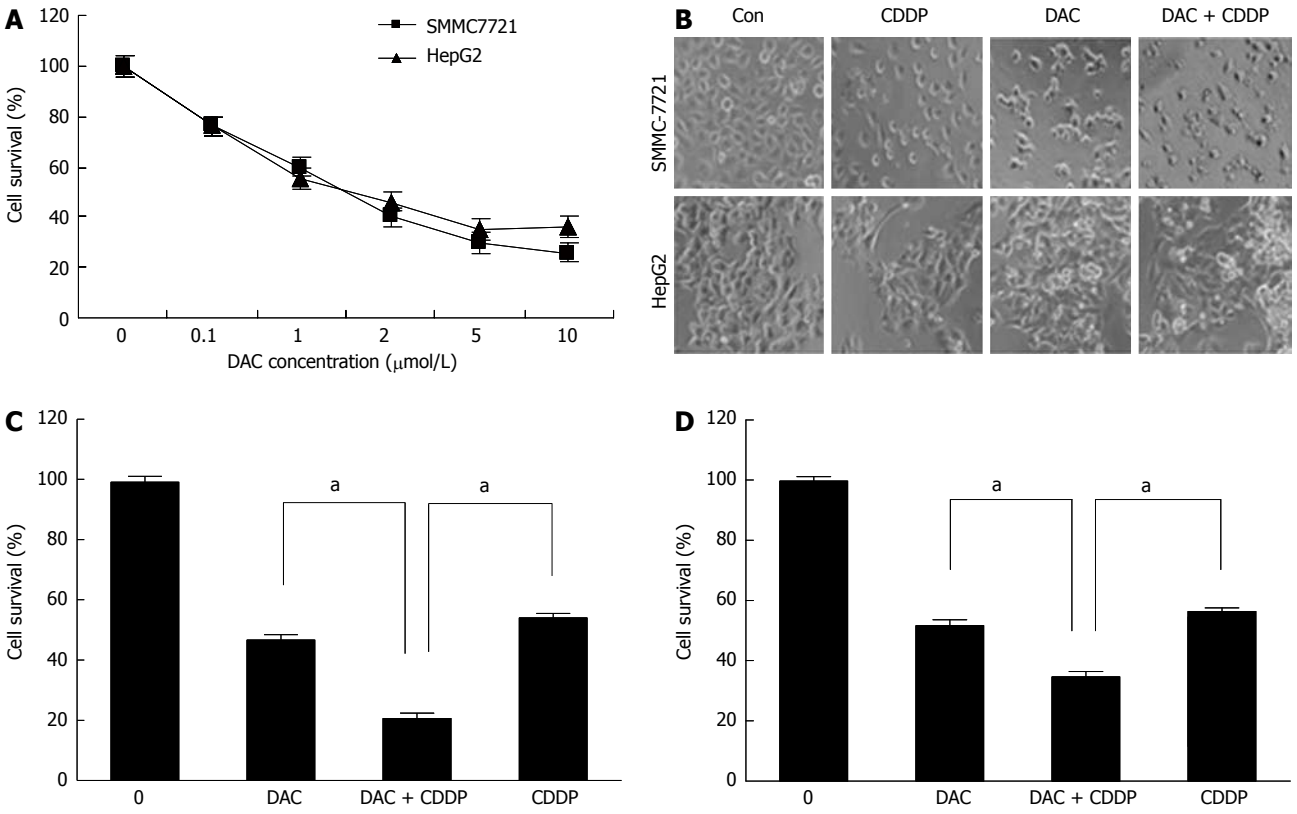

Figure 7 Sensitivity of SMMC-7721 and HepG2 cells to cisplatin enhanced by 5-aza-2'-deoxycitidine. A: Effects of DAC on growth inhibition. The hepatocellular carcinoma cell lines SMMC-7721 and HepG2 were treated with various doses of DAC for 72 h. Cell viability was determined by cell proliferation assay kit; B: The morphology of the cells was captured under a light microscope; C, D: SMMC-7721 (C) and HepG2 cells (D) cells were treated with 2 μmol/L DAC, 20 μmol/L cisplatin, or in combination for 72 h. Cell viability was determined by MTT assays. DAC: 5-aza-2'-deoxycitidine; Con: Control; CDDP: Cisplatin.

entiation, inducing demethylating agent DAC. We demonstrated that telomerase activity could be inhibited by DAC in HCC cells (HepG2 and SMMC-7721), and the demethylation of p16 promoter could play an important role.

Telomerase reactivation is a critical step in cellular immortality and carcinogenesis and is considered as a target for cancer treatment[8]. Our lab revealed that more than 85% HCC had much stronger telomerase activity than cirrhosis[16]. Drug-induced cell killing of tumor cells is associated with a decline in detectable telomerase activity[20]. In the present study, DAC inhibited telomerase activity and down-regulated hTERT expression in both human hepatoma cell lines SMMC-7721 and HepG2, and DAC suppressed the transcriptional activity of hTERT genes. Targeting telomerase activity is one of the mechanisms responsible for this reagent's inhibition of cancer cell growth.

On one hand, promoter methylation was associated with transcriptional silencing of the hTERT gene, as treat-

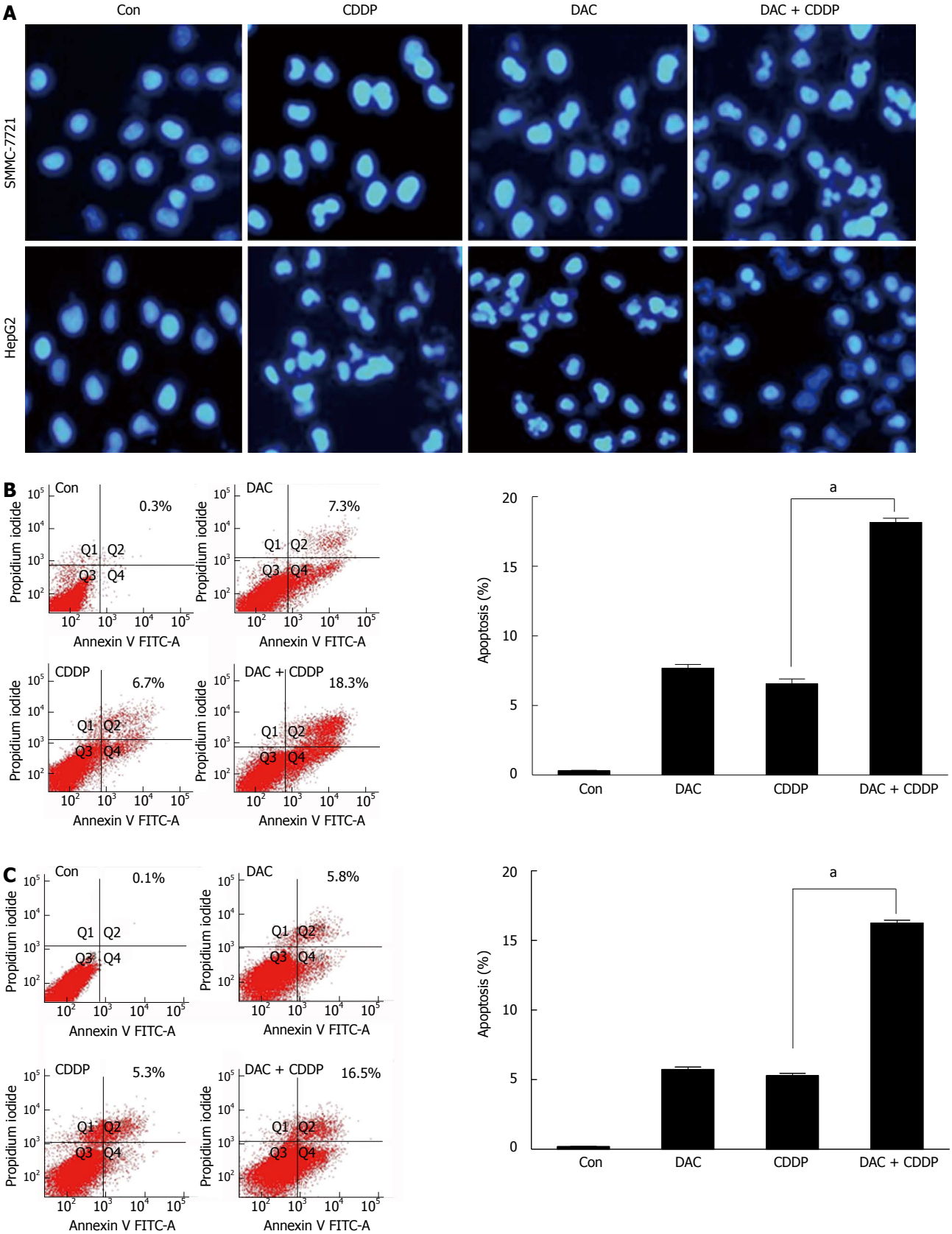

Figure 8 Apoptosis induced by 5-aza-2'-deoxycitidine. SMMC-7721 and HepG2 cells were treated with 2 μmol/L DAC, 20 μmol/L cisplatin, or in combination for 72 h. A: The fluorescent microscopic pictures of apoptotic cells were captured by 4',6-diamidino-2-phenylindole staining of the condensed and fragmented nuclei; B, C: Annexin V-fluorescein isothiocyanate (FITC) staining following facial action coding system analysis was shown for SMMC-7721 (B) or HepG2 cells (C). DAC: 5-aza-2'-deoxycitidine; Con: Control; CDDP: Cisplatin.

ment of cells with demethylating agent DAC resulted in an increase in hTERT transcription in an immortal fibroblast line SUSM-1[21]. On the other hand, DNA hypermethylation was implicated in the positive regulation of the hTERT promoter because demethylation in several telomerase-positive tumor cell lines reduced hTERT expression and telomerase activity accompanied by telomere shortening[22,23]. Our present study showed that hTERT promoter was methylated in SMMC-7721 cells, but not in HepG2 cells, and almost complete demethylation of hTERT promoter only occurred in the former cell line after DAC treatment. The explanation that CpG methylation likely interfered with the binding of transcriptional repressors, thereby positively regulating the hTERT promoter, may be reasonable for SMMC-7721 but not for HepG2. These findings suggested that hTERT promoter methylation is not the sole regulator of hTERT gene expression in HCC cells treated with DAC.

Several transcription factors are known to be responsible for the regulation of hTERT expression, including c-myc, p21, p16, p15, E2F-1 and Wilms' Tumor 1 suppressor gene[24]. This evidence prompted us to examine the effect of DAC on the expression of these genes. The c-myc was over-expressed while p16 expression was low or lost in hepatoma cell lines, and DAC repressed c-myc expression while reactivating p16. c-myc plays a critical role in telomerase activation through up-regulating the hTERT transcription, and this could be one mechanism by which 5-aza-CR represses hTERT transcription. Inactivation of p16-dependent pathways possibly in conjunction with telomerase activation might be a critical step for immortalization[25]. Our findings indicated that up-regulation of p16 and subsequent down-regulation of c-myc could be major pathways for hTERT repression by DAC.

Several evidences indicated that p16 expression could be transcriptionally silenced by CpG island hypermethylation in HCC[26]. The absence of expression and promoter methylation of p16 suggested that aberrant methylation is a major mechanism of the inactivation of p16 expression in HCC. Our data showed that DAC reversed p16 promoter methylation status and reactivated its expression, suggesting that p16 plays an important role in the down-regulation of telomerase activity by DAC.

In conclusion, the demethylating reagent 5-aza-CR represses telomerase activity and down-regulates hTERT expression. p16 could play a key role in this regulation. Our findings may provide insights into one of the mechanisms through which 5-aza-CR exerts growth-inhibitory effects on HCC cells.

COMMENTS

Background

The inactivation of tumor suppressor genes by aberrant DNA methylation plays an important role in the development of malignancies. An inhibitor of DNA methylation, 5-aza-2'-deoxycitidine (DAC), could inhibit telomerase activity in some cancer cell lines, but the molecular mechanism remains unclear.

Research frontiers

DAC can inhibit cancer cell growth, particularly leukemia cells, and it has been applied for the treatment of myelodysplastic syndromes. Targeting telomerase activity is one of the mechanisms responsible for this reagent's inhibition of cancer cell growth.

Innovations and breakthroughs

The authors demonstrated that the demethylating reagent DAC represses telomerase activity and down-regulates telomerase reverse transcriptase (hTERT) expression. p16 could play a key role in this regulation.

Applications

By understanding the mechanism of DAC repressing telomerase activity, this study may represent a future strategy for the treatment of patients with hepatocellular carcinoma (HCC).

Terminology

Telomerase and hTERT is a critical step in cellular immortality and carcinogenesis and is considered as a target for cancer treatment. And several transcription factors such as c-myc and p16 were responsible for the regulation of hTERT expression. The DNA methylation could play an important role in telomerase activity.

Peer review

This is a nice paper that describes the role of an inhibitor of DNA methylation DAC on telomerase expression and activity in HCC cell lines. Work has been well designed, effects have been proved in more than one HCC cell lines and in general terms, conclusions are supported by the presented results.

REFERENCES

1 **Patra SK**, Bettuzzi S. Epigenetic DNA-(cytosine-5-carbon) modifications: 5-aza-2'-deoxycytidine and DNA-demethylation. *Biochemistry* (*Mosc*) 2009; **74**: 613-619

2 **Fenaux P**, Ades L. Review of azacitidine trials in Intermediate-2-and High-risk myelodysplastic syndromes. *Leuk Res* 2009; **33** Suppl 2: S7-S11

3 **Buckstein R**, Yee K, Wells RA. 5-Azacytidine in myelodysplastic syndromes: a clinical practice guideline. *Cancer Treat Rev* 2011; **37**: 160-167

4 **Egger G**, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004; **429**: 457-463

5 **Altshuler ML**, Severin SE, Glukhov AI. The tumor cell and telomerase. *Biochemistry* (*Mosc*) 2003; **68**: 1275-1283

6 **Oh BK**, Kim H, Park YN, Yoo JE, Choi J, Kim KS, Lee JJ, Park C. High telomerase activity and long telomeres in advanced hepatocellular carcinomas with poor prognosis. *Lab Invest* 2008; **88**: 144-152

7 **Hytiroglou P**, Theise ND. Telomerase activation in human hepatocarcinogenesis. *Am J Gastroenterol* 2006; **101**: 839-841

8 **Kim NW**, Piatyszek MA, Prowse KR, Harley CB, West MD, Ho PL, Coviello GM, Wright WE, Weinrich SL, Shay JW. Specific association of human telomerase activity with immortal cells and cancer. *Science* 1994; **266**: 2011-2015

9 **Takakura M**, Kyo S, Kanaya T, Hirano H, Takeda J, Yutsudo M, Inoue M. Cloning of human telomerase catalytic subunit (hTERT) gene promoter and identification of proximal core promoter sequences essential for transcriptional activation in immortalized and cancer cells. *Cancer Res* 1999; **59**: 551-557

10 **Takakura M**, Kyo S, Kanaya T, Tanaka M, Inoue M. Expression of human telomerase subunits and correlation with telomerase activity in cervical cancer. *Cancer Res* 1998; **58**: 1558-1561

11 **Ito H**, Kyo S, Kanaya T, Takakura M, Inoue M, Namiki M. Expression of human telomerase subunits and correlation with telomerase activity in urothelial cancer. *Clin Cancer Res* 1998; **4**: 1603-1608

12 **Cong YS**, Wen J, Bacchetti S. The human telomerase catalytic subunit hTERT: organization of the gene and characterization of the promoter. *Hum Mol Genet* 1999; **8**: 137-142

13 **Bazarov AV**, Hines WC, Mukhopadhyay R, Beliveau A, Melodyev S, Zaslavsky Y, Yaswen P. Telomerase activation

by c-Myc in human mammary epithelial cells requires additional genomic changes. *Cell Cycle* 2009; **8**: 3373-3378
14 **Cong YS**, Wright WE, Shay JW. Human telomerase and its regulation. *Microbiol Mol Biol Rev* 2002; **66**: 407-25, table of contents
15 **Liu YC**, Chen CJ, Wu HS, Chan DC, Yu JC, Yang AH, Cheng YL, Lee SC, Harn HJ. Telomerase and c-myc expression in hepatocellular carcinomas. *Eur J Surg Oncol* 2004; **30**: 384-390
16 **Zhang C**, Guo X, Jiang G, Zhang L, Yang Y, Shen F, Wu M, Wei L. CpG island methylator phenotype association with upregulated telomerase activity in hepatocellular carcinoma. *Int J Cancer* 2008; **123**: 998-1004
17 **Zhang C**, Xu Y, Zhao J, Fan L, Jiang G, Li R, Ling Y, Wu M, Wei L. Elevated expression of the stem cell marker CD133 associated with Line-1 demethylation in hepatocellular carcinoma. *Ann Surg Oncol* 2011; **18**: 2373-2380
18 **Iliopoulos D**, Satra M, Drakaki A, Poultsides GA, Tsezou A. Epigenetic regulation of hTERT promoter in hepatocellular carcinomas. *Int J Oncol* 2009; **34**: 391-399
19 **Watanabe Y**, Maekawa M. Methylation of DNA in cancer. *Adv Clin Chem* 2010; **52**: 145-167
20 **Faraoni I**, Turriziani M, Masci G, De Vecchis L, Shay JW, Bonmassar E, Graziani G. Decline in telomerase activity as a measure of tumor cell killing by antineoplastic agents in vitro. *Clin Cancer Res* 1997; **3**: 579-585
21 **Devereux TR**, Horikawa I, Anna CH, Annab LA, Afshari CA, Barrett JC. DNA methylation analysis of the promoter region of the human telomerase reverse transcriptase (hTERT) gene. *Cancer Res* 1999; **59**: 6087-6090
22 **Guilleret I**, Yan P, Grange F, Braunschweig R, Bosman FT, Benhattar J. Hypermethylation of the human telomerase catalytic subunit (hTERT) gene correlates with telomerase activity. *Int J Cancer* 2002; **101**: 335-341
23 **Guilleret I**, Benhattar J. Demethylation of the human telomerase catalytic subunit (hTERT) gene promoter reduced hTERT expression and telomerase activity and shortened telomeres. *Exp Cell Res* 2003; **289**: 326-334
24 **Shamanin VA**, Androphy EJ. Immortalization of human mammary epithelial cells is associated with inactivation of the p14ARF-p53 pathway. *Mol Cell Biol* 2004; **24**: 2144-2152
25 **Bhatia B**, Jiang M, Suraneni M, Patrawala L, Badeaux M, Schneider-Broussard R, Multani AS, Jeter CR, Calhoun-Davis T, Hu L, Hu J, Tsavachidis S, Zhang W, Chang S, Hayward SW, Tang DG. Critical and distinct roles of p16 and telomerase in regulating the proliferative life span of normal human prostate epithelial progenitor cells. *J Biol Chem* 2008; **283**: 27957-27972
26 **Jin M**, Piao Z, Kim NG, Park C, Shin EC, Park JH, Jung HJ, Kim CG, Kim H. p16 is a major inactivation target in hepatocellular carcinoma. *Cancer* 2000; **89**: 60-68

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ORIGINAL ARTICLE

Lactobacillus crispatus M206119 exacerbates murine DSS-colitis by interfering with inflammatory responses

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Abstract

AIM: To investigate the role of *Lactobacillus crispatus* (*L. crispatus*) strain China Center for Type Culture Collection (CCTCC) M206119 in intestinal inflammation.

METHODS: Forty 8-wk-old Balb/c mice (20 ± 2 g) were divided into four groups of 10 mice each. Three groups that had received dextran sulfate sodium (DSS) were administered normal saline, sulfasalazine or CCTCC M206119 strain, and the fourth group received none of these. We assessed the severity of colitis using a disease activity index, measured the colon length and weight, collected stools and mesenteric lymph nodes for bacterial microflora analysis. One centimeter of the proximal colon, middle colon and distal colon were collected and fixed in 10% buffered formalin, dehydrated in ethanol, and embedded in paraffin. Interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α expression was detected using reverse transcription polymerase chain reaction. Protective factors zonula occludens (ZO)-1 and β-defensin 2 were detected by immunoblotting. The features of CCTCC M206119 strain were identified based on morphology, biochemical profile, and 16S RNA sequencing.

RESULTS: DSS-colitis animals treated with CCTCC M206119 had markedly more severe disease, with greater weight loss, diarrhea, fecal bleeding, and shortened colon length. In addition, the CCTCC-M206119-treated group had comparatively higher histological scores and more neutrophil infiltration than the controls. Expression of protective factors ZO-1 and β-defensin 2 was downregulated due to destruction of the mucosal barrier after CCTCC M206119 strain treatment. An *in vitro* assay demonstrated that CCTCC M206119 strain increased the nuclear translocation of nuclear factor-κB in epithelial cells. Intestinal proinflammatory or anti-inflammatory cytokine responses were evaluated. Proinflammatory colonic cytokine (IL-1β, IL-6 and TNF-α) levels were clearly increased in CCTCC-M206119-treated animals, whereas anti-inflammatory colonic cytokine (IL-10) level was lowered compared with saline or 5-aminosalicylic-acid-treated DSS-colitis mice. Next, CCTCC M206119 strain was characterized as *L. crispatus* by microscopic morphology, biochemical tests and 16S rRNA gene level.

CONCLUSION: Not all lactobacilli are beneficial for intestinal inflammation, and *L. crispatus* CCTCC M206119 strain is involved in exacerbation of intestinal inflammation in DSS-colitis mice.

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Key words: Colitis; *Lactobacillus crispatus*; Intestine; Dextra sodium sulfate; Mice

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INTRODUCTION

Inflammatory bowel diseases (IBD), including Crohn's disease and ulcerative colitis (UC), are characterized by an abnormal activation of the gut-associated immune system resulting in a chronic inflammation of the digestive tract[1]. It is widely accepted that a combination of genetic factors, immune disorders and environmental factors could be involved in the etiology of IBD[2]. Recent research has shown that some commensal and pathogenic bacteria are closely related to these diseases[3,4].

In acute dextran sulfate sodium (DSS)-induced colitis, bacteria and/or bacterial products play a major role in the initiation of inflammation but not in chronic DSS-colitis[5]. In the neonatal period, *IL-10* gene-deficient mice have decreased levels of colonic *Lactobacillus spp.* and an increase in colonic mucosal adherent and translocated bacteria. Normalizing *Lactobacillus spp.* levels reduces colonic mucosal adherent and translocated bacteria and prevents colitis[6]. Also, it is widely accepted that antibiotic treatment can definitely alleviate the clinical manifestation of UC. Various antibiotics have been shown to exhibit varying abilities to prevent and treat colitis in HLA-B27 rats and *IL-10*$^{-/-}$ mice[7,8]. Some authors recommended that where there are no bacteria, there is no colitis. Various IBD models in rodents reared under germ-free conditions are free of intestinal inflammation[6]. Increasing evidence supports that luminal microflora, or their products, are probably an important initiating factor in the pathogenesis of IBD[9].

Interestingly, facultative anaerobic Gram-positive bacteria and *Lactobacillus spp.* are decreased in patients with active UC, whereas bifidobacteria are decreased in fecal extracts from patients with Crohn's disease (CD)[10]. Another group has shown that bifidobacteria are reported to be deficient in rectal biopsies from patients with IBD and studies of fecal flora in patients with CD suggest a deficiency in both lactobacilli and bifidobacteria[11]. Furthermore, a marked decrease in intestinal *Lactobacillus spp.* precede the onset of colitis in *IL-10* gene deficient mice, and specific lactobacilli are relevant to disease induction or progression in the IL-10-deficient mouse model of colitis[12]. These studies offer strong evidence to support the view that an imbalance in the intestinal microflora triggers intestinal inflammation.

Microflora considered beneficial to the host includes the genera *Bifidobacterium* and *Lactobacillus*, whereas species potentially pathogenic include Enterobacteriacae and clostridia[4]. Therefore, maintaining or recovering the intestinal microflora or mucosa-associated bacteria in specific compartments has been proposed as a potential therapy to treat IBD[13]. For example, the administration of the VSL#3 probiotic cocktail delays relapse of pouchitis after surgical resection[14,15]. Probiotics have undergone investigation for their capacity to reduce the severity of a number of inflammatory conditions including pouchitis and UC. Also, some natural anti-inflammatory effects have recently been shown for *Lactobacillus salivarius*, *Lactobacillus crispatus* (*L. crispatus*), *Bifidobacterium* and *Lactobacillus plantarum* and *Lactobacillus casei* Shirota based on experimental colitis models[16-18].

Despite the wide usage of probiotics in controlling IBD, their efficacy is still controversial[19,20]. Thus, it is very difficult to draw a definitive conclusion in evaluating the role of microflora in pathogenesis of IBD, and subsequently, the efficacy of controlling IBD. Some published data have also questioned the safety of probiotics. The possibilities include possible adverse effects of probiotics in diseased or debilitated patients, rational bacterial selection, bacterial translocation, and microflora shift among individuals. Some published data have shown that lactobacilli isolated from IL-10-deficient mice failed to decrease tumor necrosis factor (TNF)-α production, whereas six lactobacillus isolates from mice without colitis significantly inhibited TNF-α production. This indicates that multiple strains of lactobacteria may be capable of probiotic activity but, conversely, that not all strains of lactobacteria have *in vitro* immunomodulatory activity[12]. Interestingly, it has been reported that *Lactobacillus rhamnosus* GG exacerbated acute DSS-induced colitis, demonstrated by increased colitic disease activity and histological damage score, however, in a chronic colitis model, a protective effect was observed[21].

In a previous study, we investigated and compared the effect of different *Lactobacillus* and *Bifidobacterium* strains, in a model of DSS-induced colitis[22]. When we screened to find some probiotics that can be potentially therapeutic, we came across a strain *L. crispatus* China Center for Type Culture Collection (CCTCC) M206119 that is capable of aggravating murine DSS-colitis. What was the possible mechanism? Does it mean that probiotics safety should be reconsidered?

In the present study, the promotive effects of strain CCTCC M206119 on the development of the inflammation were analyzed using a DSS-colitis model in BALB/c mice. We compared the effect of CCTCC M206119 administration, saline, and 5-aminosalicylic acid (5-ASA) treatment on DSS-colitis in mice. Thus, we tested *in vivo* and *in vitro* whether and how the CCTCC M206119 strain could aggravate epithelial damage of the colon, and indentified the features of CCTCC M206119 strain based on morphology, biochemical profiles, and 16S RNA sequencing.

MATERIALS AND METHODS

Bacterial strains and growth conditions

L. crispatus strain CCTCC M206119 was isolated from

Table 1 Experimental protocol for induction of dextran sulfate sodium-colitis

Groups	Experimental design
Healthy control	No DSS treatments
Negative control (saline)	5% (w/v) DSS dissolved in drinking water for 7 d
	Saline administered intragastrically once daily for 9 d
Positive control (5-ASA)	5% (w/v) DSS dissolved in drinking water for 7 d
	5-ASA administered intragastrically once daily for 9 d
Investigating group (CCTCC M206119)	5% (w/v) DSS dissolved in drinking water for 7 d
	CCTCC M206119 administered intragastrically once daily for 9 d

DSS: Dextran sulfate sodium; 5-ASA: 5-aminosalicylic acid.

human feces in the Division of Digestive Disease of the Second XiangYa Hospital (Changsha, Hunan, China), and kept at China Center for Type Culture Collection (CCTCC, Wuhan, China). *Lactobacillus* strains were grown at 37 ℃ in de Man, Rogosa and Sharpe (MRS) medium (Difco, MD, United States) without shaking.

Animals

Eight-week-old BALB/c mice of either sex were purchased from Hunan Agricultural University [SCXK (Xiang 2002-003)], and then bred under specific pathogen-free conditions. Mice were kept in ventilated and filtered cages, fed an irradiated diet, and housed on irradiated bedding. Food and water were supplied *ad libitum*. All animal experiments were performed in compliance with the guidelines of the Chinese government approved by the College BioResources ethical review board.

Preparation of bacterial strains and administration to mice

Bacterial strains were grown to an optical density at 600 nm of 3 to 4 (early stationary phase) at 37 ℃ in MRS medium anaerobically, harvested by centrifugation, washed with sterile neutral saline, and resuspended at 10^9 colony forming units (CFU)/mL in neutral saline. Three hundred microliters of this daily-prepared suspension was fed daily to the mice *via* intragastric gavage, whereas the controls (both UC-induced and healthy mice) received 300 μL neutral saline *via* the same procedure.

DSS-induced colitis model and experimental design

Eight-week-old Balb/c mice (20 ± 2 g) were divided into a healthy control group, negative control (normal saline) group, positive control (5-ASA, 6 mg/20 g) group, and investigation (CCTCC M206119 strain) group, with 10 mice in each. Normal saline (300 μL), sulfasalazine, CCTCC M206119 strain were administered intragastrically once daily for 2 d before starting DSS and continued for 7 d after DSS induction. Colitis was induced by 5% (w/v) DSS (molecular weight 35 000-50 000; MP Biomedicals, Solon, OH, United States) dissolved in drinking water for 7 d (Table 1). The healthy control group received no DSS. Severity of colitis was assessed daily using a disease activity index (DAI). After the seventh day of induction of colitis, animals were euthanized by CO_2 asphyxiation. The colon lengths and weight were measured. Under aseptic technique, 300 mg stools and mesenteric lymph nodes (MLNs) were collected for bacterial microflora analysis. All the samples were placed immediately in sterile tubes containing 5 mL of transport medium. One centimeter of the proximal colon, middle colon and distal colon was collected and fixed in 10% buffered formalin, dehydrated in ethanol, and embedded in paraffin. The remaining tissues were immediately snap-frozen and stored at -80 ℃[23,24].

Table 2 Scoring of disease activity index

Score	Weight loss (%)	Stool consistency	Occult/gross fecal bleeding
0	0	Normal	Negative
1	1-5		
2	5-10	Loose stool	Hemoccult
3	10-15		
4	> 15	Diarrhea	Gross bleeding

DAI = (combined score of weight loss, stool consistency, and bleeding)/3. Normal stool, well-formed pellets; Loose stool, pasty and semi-formed stool that does not adhere to the anus; Diarrhea, liquid stool that adheres to the anus.

Table 3 Histological score to quantify the degree of colitis

Score	Inflammation	Depth of lesions	Destruction of crypt	Width of lesions (%)
0	None	None	None	
1	Mild	Submucosa	1/3 basal crypt	1-25
2	Severe	Muscularis	2/3 basal crypt	26-50
3		Sera	Intact epithelium only	51-75
4			Total crypt and epithelium	76-100

Assessment of colitis DAI

The severity of colitis was assessed daily using a DAI based on the scoring system of Hamamoto *et al*[25], which scores body weight loss, stool consistency and occult/gross fecal bleeding (Table 2). Occult blood in feces was evaluated by means of Hemoccult II test (Beckman Coulter, Palo Alto, CA, United States).

Histology and histological grading

Tissue fixed with 4% (w/v) paraformaldehyde in phosphate buffered solution (PBS) was prepared for light microscopy, and 5-μm-thick sections were stained with hematoxylin and eosin to study histological changes. Grading of intestinal inflammation was determined as follows (Table 3)[26]. All scores were obtained in a blinded fashion by two independent investigators.

Table 4 Primers used to detect gene expression at mRNA level

Genes		Sequence of primers
GAPDH	Sense	5′-ATCACCATCTTCCAGGAGCG-3′
	Anti-sense	5′-CCTGCTTCACCACCTTCTTG-3′
IL-1β	Sense	5′-TTTTAATCAGCTATCCGGAC-3′
	Anti-sense	5′-TAATGGGAACGTCACACACC-3′
IL-6	Sense	5′-ATGAAGTTCCTCTCTGCAAGAGACT3′
	Antisense	5′-CACTAGGTTTGTTTAATCTC-3′
TNF-α	Sense	5′-ACGTGGAACTGGCAGAAGAG-3′
	Antisense	5′-GGTTGTCTTTGAGATCCATGC-3′

Analysis of colon microflora

Three-hundred-microgram fecal samples were dispersed in 2 mL PBS. Each pooled sample (0.1 mL) was serially diluted *via* 10-fold dilutions (from 10^{-1} to 10^{-10}). Eosin methylene blue agar, KULB agar, TTC azide dextrose agar and mannitol salt agar were used for the enumeration of *Enterobacteriaceae*, *Bacteroides*, *Enterococcus* and *Staphylococcus spp.*, respectively. Standard Nutrient Agar was used for the enumeration of total aerobic bacteria. All the plates were incubated at 37 ℃, aerobically, for 24-48 h and the number of colonies were counted. Brain-heart infusion agar and LAMVAB agar were used to enumerate bifidobacteria and lactobacteria, respectively. Anaerobic incubation was carried out in anaerobic jars (Oxoid, Basingstoke, Hants, United Kingdom) at 37 ℃ for 48-72 h. Anaerobic conditions were obtained using Anaerogen (Oxoid) and were checked using methyl blue strips as oxidation reduction indicator. CFUs were counted and expressed per gram of sample[27,28].

Myeloperoxidase activity

The extent of neutrophil infiltration was determined by myeloperoxidase (MPO) assay[29]. Briefly, 500 mg colon was homogenized in 1 mL iced 0.5% hexadecyltrimethylammonium bromide buffer. The aliquot was centrifuged (4000 *g*, 15 min) and the supernatant was diluted into 5% homogenates. A mixture of 0.1 mL 0.05% hydrogen peroxide and 0.9 mL 5% homogenates was vortexed to release MPO from the tissue. After incubation in 37 ℃ for 15 min, 0.2 mL mixture was added to 3 mL O-dianisidine reaction mixture and incubated for 30 min at 37 ℃. Sodium azide (50 μL, 2.0%) was added and incubated at 60 ℃ for 10 min. Absorbance of the reaction mixture was measured at 460 nm by spectrophotometer. Saline was used as a control. MPO activity was expressed as U/g tissue.

Nuclear translocation of nuclear factor-κB in CCTCC-M206119-stimulated HT-29 cells

HT-29 cells (purchased from Y-Y Chemical Reagent Co. Ltd., Shanghai, China) were cultured in RPMI 1640 medium (Gibco, Rockville, MD, United States) supplemented with 10% fetal calf serum under 5% CO_2 at 37 ℃ and divided into negative control, TNF-α (10 ng/mL) and CCTCC M206119 groups. CCTCC M206119 was diluted to 10^9 CFU/mL with RPMI 1640 medium. HT-29 (3×10^6 cells) were cultured in 24-well tissue culture plates for 72 h and TNF-α or CCTCC M206119 was added. After 4 h of incubation, HT-29 cells were fixed in cold methanol for 5-15 min. The fixed cells were blocked in 5% bovine serum albumin (BSA)/PBS for 1 h on ice. We remove the blocking buffer and added primary antibody anti-nuclear factor (NF)-κB p65 (10 μg/mL) (MBL, Woods Hole, MA, United States; clone 8F11, rat IgG2a) diluted in PBS/0.3% Triton X100/1%BSA/1% serum overnight at 4 ℃ in a humidified chamber. After washing three times for 10 min with PBS on ice, the cells were incubated with anti-rabbit Cy3 (10 μg/mL) (Abcam, Cambridge, United Kingdom; polyclonal rabbit IgG) for 1 h at room temperature. The cells were mounted in Vectashield mounting medium (Vector Laboratories, Burlingame, CA, United States) with 4',6'-diamidino-2-phenylindole hydrochloride after three washes in PBS at room temperature, and analyzed by a Nikon ECLIPSE E600 fluorescent microscope. The ratio of positive nuclear cells to total cells represented the nuclear translocation of NF-κB. The average number of positive stained cells within at least nine independent high power fields (400 × magnification) were determined microscopically and subjected to statistical analysis as indicated.

Reverse transcription polymerase chain reaction

Total tissue RNA was isolated using TRIzol reagent following the manufacturer's instructions For each sample, first-strand cDNA was synthesized using 1.0 μg total RNA with oligo dT23VN primer and M-MuLVuLV reverse transcriptase (Gibco). Five milliliters cDNA samples were amplified in 25 μL of a reaction mixture containing 10 × Taq buffer, 1.5 mmol $MgCl_2$, 5 μmol dNTPs (each), 10 μmol of each 5' and 3' primers, and 2 U TaqGold polymerase (Perkin-Elmer Cetus, Waltham, MA, United States). Polymerase chain reaction (PCR) was performed in a thermal cycler (GeneAmp Model 2400; Perkin-Elmer Cetus) for 22 cycles (94 ℃ for 30 s, 55 ℃ for 30 s, and 72 ℃ for 1 min), followed by a 10-min extension at 72 ℃. The PCR products (5 μL) were subjected to electrophoresis on 1.5% agarose gels and stained with 0.5 μg/mL ethidium bromide. Primers used in this paper are illustrated in Table 4.

Immunoblotting

Colon extracts (40 μg total protein) from each group of mice were resolved by SDS-PAGE under reducing conditions and transferred to a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA, United States). The membrane was blocked with 5% nonfat dry milk overnight and incubated with a rabbit polyclonal antibody to zonula occludens (ZO)-1, β-defensin 2 (Lab Version, Fremont, CA, United States), with a rabbit polyclonal antibody to IL-1β, IL-6 or TNF-α (Santa Cruz Biotechnology, Santa Cruz, CA, United States), with a mouse monoclonal antibody to IL-10 (BD Biosciences, Franklin

Lakes, NJ, United States), or with an anti β-actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody (Affinity BioRegents, Golden, CO, United States) for loading control. Antigen-antibody binding was detected with horseradish-peroxidase-conjugated anti-rabbit or mouse IgG (Vector Laboratories) using enhanced chemiluminescence (Amersham Biosciences, Piscataway, NJ, United States).

Scanning electron microscopy

To investigate the characterization of CCTCC M206119 strain, 10^8 CFU/mL bacteria were grown for 18 h in MRS (Difco), washed with PBS and fixed with 2.5% glutaraldehyde. After two washings with PBS, samples were dehydrated with ethanol to the critical point, coated with 20 nm of gold in the scanning electron microscopy (SEM) coating unit, and examined by a XL30 ESEM scanning electron microscope (Philips, Eindhoven, Netherlands).

Biochemical profiling

CCTCC M206119 strain were grown on MRS agar and incubated under anaerobic conditions at 37 ℃ for 24-48 h. All isolates were visualized by Gram staining. Biochemical testing was performed with API 20A strips (BioMerieux, Hazelwood, MO, United States) according to the manufacturer's instructions. Catalase and SPOR tests were performed according to the supplier's recommendations (Becton Dickinson). G + C percentage was determined using high-performance liquid chromatography (HPLC) method in the Chemistry College of Central South University (China). Ten microliters base standard and 10 μL DNA hydrolase were injected into the HPLC sample injector and analyzed three times. The separating conditions used were as follows: 250 mm Zonbax-C18 column, 0.05 mol/L $NH_4H_2PO_4$ (pH 4.0) and acetonitrile mixture (20:1) as washing solution, 1 mL/min, moving phase: 2% acetonitrile, 98% 50 mmol/L NaH_2PO_4 buffered solution (pH 3.94), room temperature, detecting wavelength 254 nm and 270 nm, 0.1 mg/mL dA, dT, dG, dC solution used as external standard.

16S rRNA gene sequencing

Approximately 700 bp of the *16S rRNA* gene were amplified with primers 16S-F (5'-AGA GTT TGA TCA TGG CTC AG-3') and 16S-R (5'-CAC CGC TAC ACA TGG AG-3') under the following PCR conditions: 95 ℃ for 5 min; 10 cycles of 94 ℃ for 30 s, 70 ℃ for 30 s, and 72 ℃ for 40 s; 26 cycles of 94 ℃ for 30 s, 60 ℃ for 30 s, and 72 ℃ for 1 min; and 72 ℃ for 10 min. 16S rDNA amplicons were gel purified by using SDS-PAGE and a gel band purification kit (Amersham Biosciences). The 5' terminus of the *16S rRNA* gene was sequenced with primers 16S-F and 16S-R by using an ABI Prism 3100 (Applied Biosystems) sequencing system and an ABI Prism BigDye Terminator cycle sequencing ready reaction kit (version 2.0, Applied Biosystems). Sequencing traces of amplicons containing ambiguous signals were resubmitted for sequencing. rDNA sequences were analyzed by using Lasergene (version 5.0, DNAStar, Madison, WI, United States). Contigs were generated by using SeqMan. Isolates were identified by using the nucleotide-nucleotide Basic Local Alignment Search Tool (BLASTn) (www.ncbi.nlm.nih.gov/BLAST).

Statistical analysis

Data were analyzed by Student's *t* test or analysis of variance (ANOVA). The Wilcoxon signed-rank test was used to compare data that did not satisfy Student's *t* test. $P < 0.05$ was considered significant.

RESULTS

Colonic inflammation was aggravated in DSS-colitis mice after CCTCC M206119 treatment

Survival rate of DSS-colitis mice reduced to 80% from day 5 after CCTCC M206119 treatment changes, which differed from other groups significantly (Figure 1A). Body weight loss, stool consistency, and occult/gross fecal bleeding were evaluated and scored individually for each animal according (Table 2). The scores were evaluated statistically by χ^2 test and Monte Carlo exact test. CCTCC-M206119-treated DSS-colitis animals showed much higher DAI scores compared with 5-ASA- and saline-treated DSS-colitis mice, starting from day 3 after 5% DSS treatment (Figure 1B).

After 7 d of treatment, all DSS-colitis mice showed a significant reduction in their colon length compared with the healthy controls. CCTCC-M206119-strain-treated DSS-colitis mice had the shortest length (8.2 ± 0.68 cm), followed by saline- (9.8 ± 0.56 cm) or 5-ASA- (10.0 ± 0.89 cm) treated DSS-colitis mice ($P < 0.05$, Figure 1C). CCTCC-M206119-treated DSS-colitis mice had markedly more severe disease, with greater weight loss, diarrhea, fecal bleeding, and shortened colon length.

The histological lesions were examined blindly and the average scores were evaluated statistically by ANOVA. In the acute study, CCTCC-M206119-treated DSS-colitis mice presented scores of 10.9 ± 0.5 that differed statistically from those in the 5-ASA-treated DSS-colitis mice. It became evident that the CCTCC-M206119-treated DSS-colitis group had comparatively higher DAI scores than the saline controls ($P < 0.01$, Figure 1D and E).

Furthermore, there was significant difference in MPO enzymatic activity detected in the colons control mice (1.038 ± 0.012 U/g tissue), DSS plus saline group (18.368 ± 1.226 U/g tissue), DSS plus 5-ASA group (8.369 ± 0.652 U/g tissue), and DSS plus CCTCC M206119 group (24.565 ± 2.006 U/g tissue) (Figure 1F). This indicated that more neutrophils infiltrated DSS-colitis mice after CCTCC M206119 treatment.

CCTCC M206119 administration changed the fecal microflora in DSS-colitis mice

To characterize the role of CCTCC M206119 strain on potential gut flora shifts during DSS-induced barrier damage, we performed a bacterial-culture-based survey

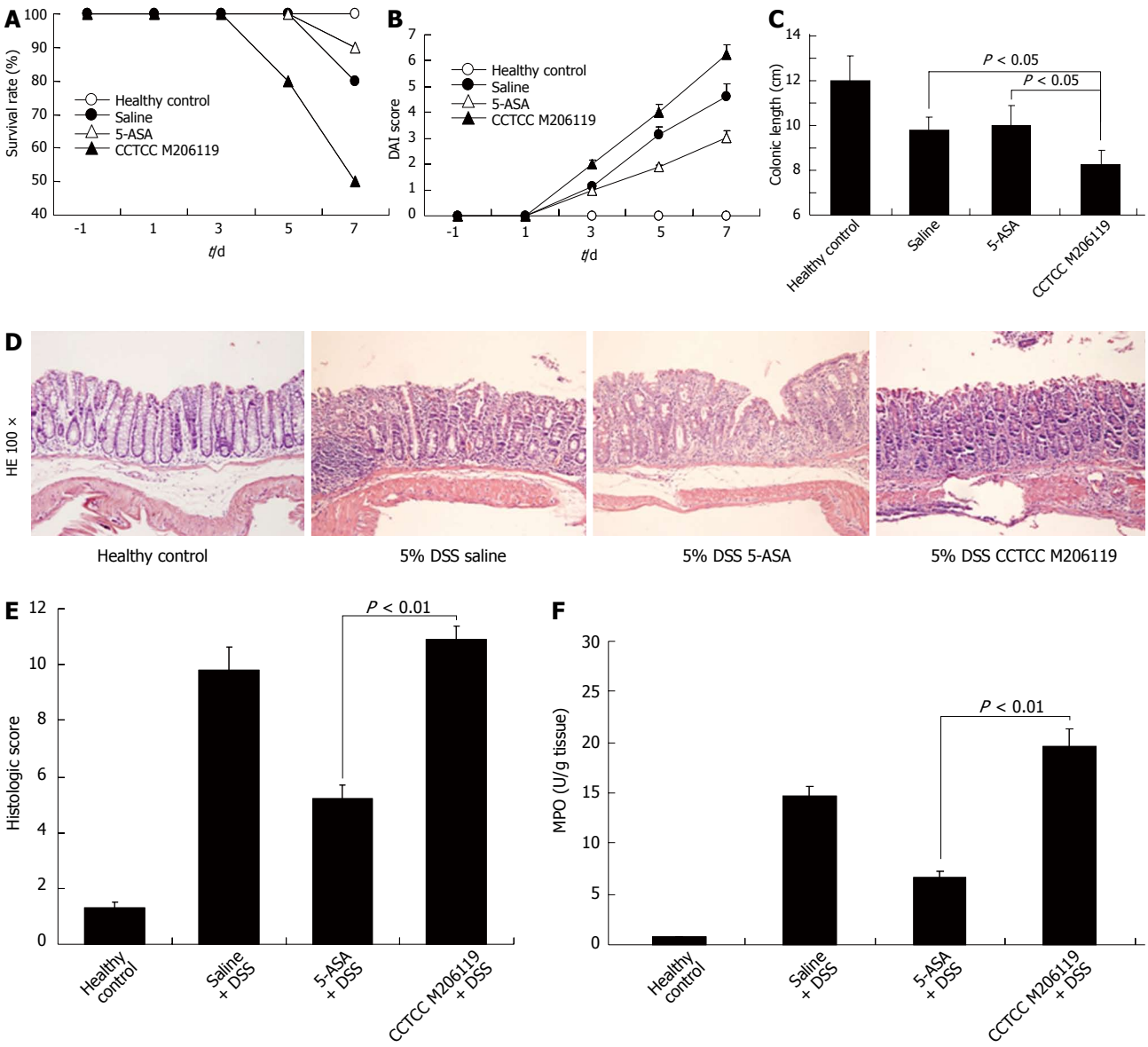


Figure 1 Colonic inflammation was aggravated in dextran sulfate sodium-colitis mice after China Center for Type Culture Collection M206119 treatment. A: Survival rate changes of four age- and sex-matched BALB/c mouse groups (n = 10) [○ : Healthy controls; ● : 5% dextran sulfate sodium (DSS) plus saline treatment; △ : 5% DSS plus 5-aminosalicylic acid (5-ASA) treatment; ▲ : 5% DSS plus China Center for Type Culture Collection M206119] at the indicated time points; B: Disease activity index (DAI) changes among groups in (A) after 7 d 5% DSS treatment at the indicated time points. Each point represents the mean ± SE (n = 10); C: Mean colon length (cm) for the mice in (A); D: Histological analysis of representative colons from the mice in (A) (original magnification, hematoxylin and eosin staining, 100 ×); E: Summarized histological scores from colons of (D); F: Summarized myeloperoxidase (MPO) activities of colons from mice in (D).

of the gut flora in DSS-colitis mice treated with saline, 5-ASA, or CCTCC M206119 strain. No significant differences in total aerobic bacteria or *Enterococcus* were obtained for any of the groups. Bacterial counts of all fecal aerobic [*Escherichia coli* (*E. coli*), *Staphylococcus*, *Bacteroides*, or total aerobic bacteria] cultured in DSS-colitis mice increased significantly compared to the healthy group. Also, both *E. coli* and *Staphylococcus* increased in the saline group, whereas 5-ASA rebalanced the bacterial counts of *Staphylococcus* to normal levels (Figure 2A). In contrast, *Lactobacillus* in the colon lumen was reduced significantly in all DSS-colitis groups, with no significant changes in *Bifidobacterium* or *Enterococcus spp.* (Figure 2B).

To determine whether the changes in gut flora after CCTCC M206119 treatment were due to alteration of the epithelial junction or antagonistic agents, we measured the expression levels of ZO-1 and β-defensin 2 from colons of DSS-colitis mice treated with saline or CCTCC M206119 and healthy mice, by western blotting. Expression of ZO-1 in healthy mice remained at an abundant level compared to all DSS-colitis mice. Treatment of mice for 5 d with 5% DSS plus saline reduced ZO-1 expression significantly compared to healthy mice, whereas 5% DSS plus CCTCC M206119 strain reduced ZO-1 expression dramatically at day 3. In contrast, no β-defensin 2 expression was detected in the healthy mice, with relatively high expression of β-defensin 2 detected in the colons in mice that were treated with 5 d of either

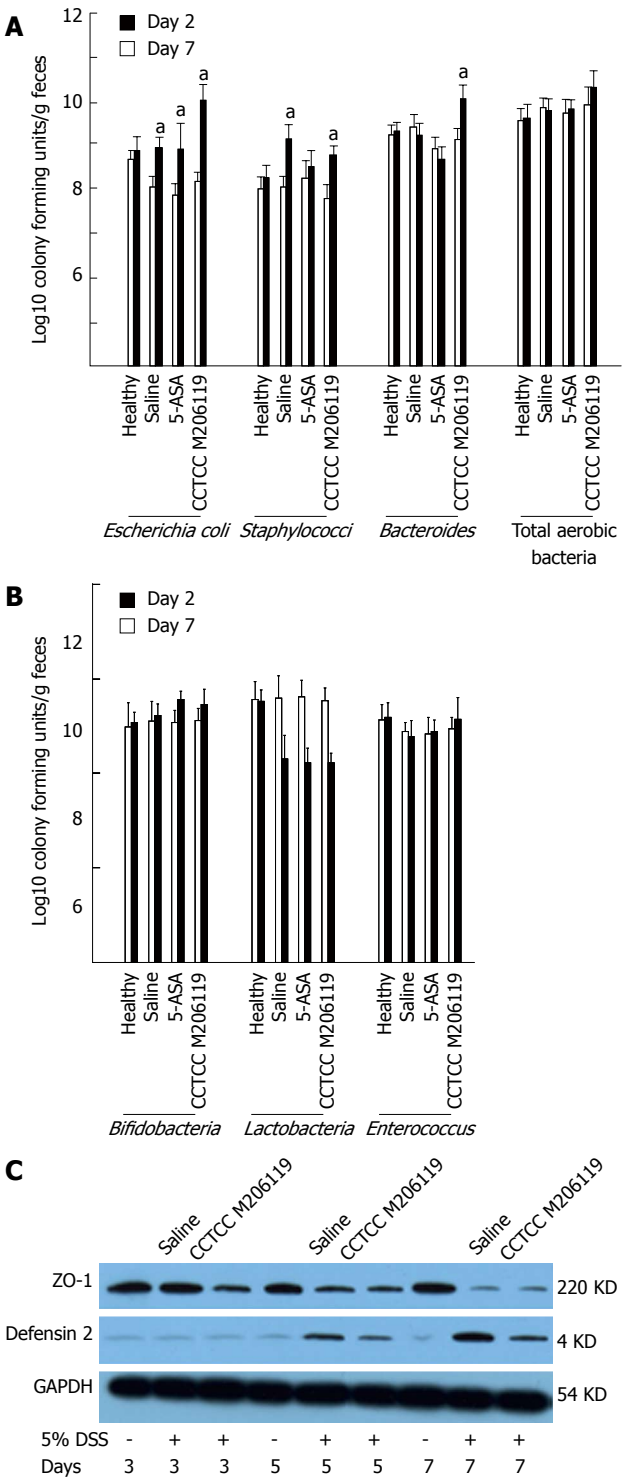


Figure 2 Alteration of fecal microflora in DSS-colitis mice after China Center for Type Culture Collection M206119 treatment. Bacterial counts of (A) aerobic (*Escherichia coli*, *Staphylococcus*, *Bacteroides*, total aerobic bacteria) and (B) anaerobic (*Bifidobacterium*, *Lactobacillus*, *Enterococcus spp.*) in colon luminal contents from healthy animals and dextran sulfate sodium (DSS)-colitis mice treated by saline, 5-aminosalicylic acid (5-ASA), or China Center for Type Culture Collection (CCTCC) M206119 strain at day 2 and day 7 after DSS-treatment, as determined by culture. The data sets were pooled from two independent experiments. Significance levels were determined by *U* test. Underlined *P* values shown in black were calculated from comparisons of healthy *vs* diseased animals; C: Zonula occludens (ZO)-1 and defensin 2 expression from colons of DSS-colitis mice treated with saline or CCTCC M206119 and healthy mice was measured by western blotting at indicated time points; GAPDH was used as a loading control. The data represent at least three experiments. [a]$P < 0.05$ *vs* healthy group. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

5% DSS plus saline or 5% plus CCTCC M206119. After 5 d exposure to 5% DSS plus CCTCC M206119, there was a significant ($P < 0.05$) decrease in β-defensin 2 level in the colons of mice relative to 5% DSS plus saline treatment (Figure 2C).

Effects of CCTCC M206119 strain on epithelial injury and translocation of NF-κB in vitro

Nuclear translocation of NF-κB p65 represents increased inflammation at the initiation stage. To investigate whether the aggravation of DSS-colitis after CCTCC M206119 strain treatment was due to the initiation of inflammation-related transcription, we detected the translocation of NF-κB p65 in HT-29 cells inoculated with CCTCC M206119 strain. TNF-α was used as a control. In the blank control, no positive staining for NF-κB p65 was detected. Both TNF-α and CCTCC M206119 strain upregulated the translocation of NF-κB p65 *in vitro*, with more positively stained cells in the latter group ($P < 0.05$, Figure 3A and B).

Colonic cytokines in CCTCC-M206119- or vehicle-treated DSS-colitis mice

To investigate if CCTCC M206119 administration affected the expression of colonic cytokines, we first screened the mRNA levels of the major colonic proinflammatory cytokines by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR). We observed increased mRNA levels for IL-1 β, IL-6 and TNF-α from the colons of DSS-colitis mice treated with CCTCC M206119 strain or saline for 3 d. Also, it was evident that mRNA levels of IL-1 β, IL-6 and TNF-α from colons of CCTCC-M206119-treated DSS-colitis mice were significantly higher than those from colons of saline-treated DSS-colitis mice (Figure 4A). Next, we confirmed the changes in expression of the above cytokines at the protein level, suggesting that DSS treatment resulted in increased expression of proinflammatory cytokines, whereas DSS plus CCTCC M206119 strain treatment resulted in significantly higher expression of the cytokines mentioned above (Figure 4B).

Anti-inflammatory cytokine IL-10 expression from colons of DSS-colitis mice treated with saline, 5-ASA, or CCTCC M206119 was measured by western blotting. IL-10 expression was dramatically downregulated in saline- and CCTCC-M206119-treated DSS-colitis mice, with more significant changes in the latter group. In contrast, 5-ASA upregulated IL-10 expression compared to saline-treated DSS-colitis mice (Figure 4C). It indicated that CCTCC M206119 treatment inhibited the expression of anti-inflammatory cytokine in DSS-colitis mice.

Characteristics of CCTCC M206119 strain

Distinct microscopic morphological features were observed after Gram staining of broth-grown CCTCC M206119 strain. Gram-positive, rod-shaped morphology could be distinguished (Figure 5A). Under SEM, CCTCC M206119 strain showed exclusively smooth rod-

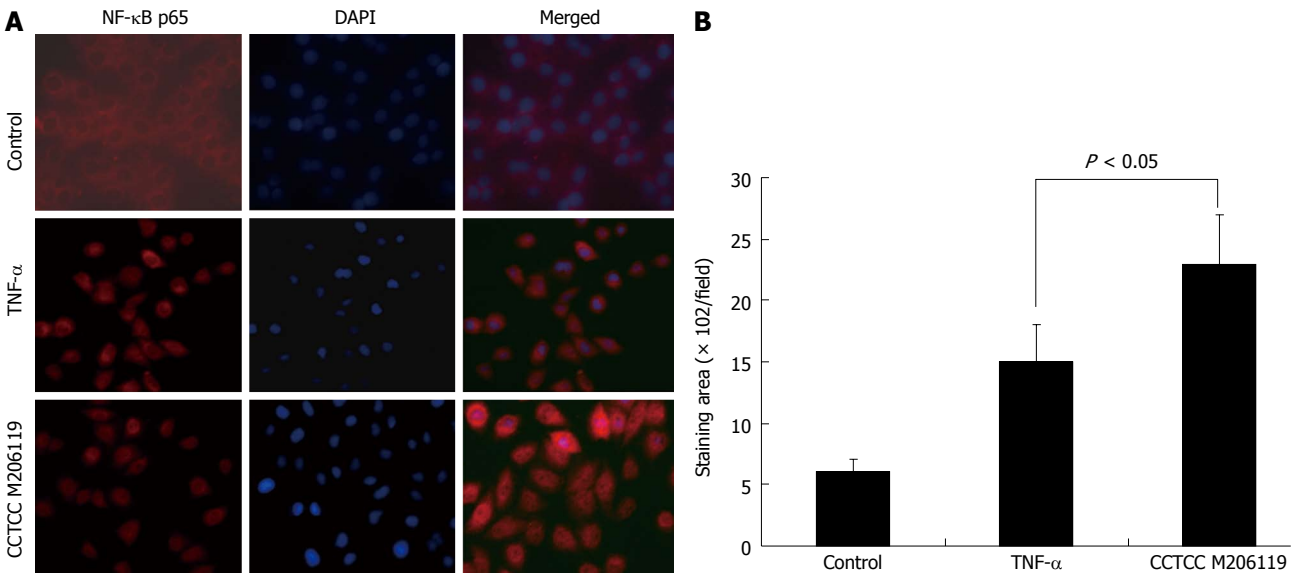


Figure 3 Effects of China Center for Type Culture Collection M206119 strain on epithelial injury and translocation of nuclear factor-κB *in vitro*. Immunofluorescence staining for nuclear factor (NF)-κB p65 (red) and 4',6'-diamidino-2-phenylindole hydrochloride (DAPI) (blue) in HT-29 cells treated either by tumor necrosis factor (TNF)-α or China Center for Type Culture Collection (CCTCC) M206119 (scale bars, 10 mm). A: Increased positive NF-κB p65 staining after CCTCC M206119 treatment; B: Positive staining areas were quantified as pixels per high-power microscopic field (400 ×) using Photoshop. Error bars indicate mean ± SD (n = 3).

or fork-shaped morphology, without spores, flagella or capsules (Figure 5A). Based on analysis of the peak and data of base C, G, T and A, G + C mol% of CCTCC M206119 strain was determined as 58.64% (Figure 5B). All CCTCC M206119 strains tested were unable to utilize indepamide, urea enzymes and gelatin. All strains tested were able to utilize glucose, mannitol, lactose, saccharose, maltose, salicin, L-xylose, arabitol, esculin, glycerol, cellobiose, MNC, melezitose, D-raffinose, sorbitol, rhamnose, and trehalose. All strains were found to be catalase negative [at 3% (v/v) H_2O_2]. With discriminant and factorial analyses of data from all biochemical tests, a presumptive identification scheme for CCTCC M206119 strain was formulated based on biochemical properties. In conjunction with Gram stain morphology, biochemical profiling can be used to differentiate among major groups of lactobacilli (up to 95% confidence interval). In our abbreviated identification scheme, CCTCC M206119 strain can be presumptively grouped into lactobacilli when biochemical tests are combined with microscopic morphology (Figure 5C). To verify the identities of the reference strains used in this study, 16S rRNA genes were amplified and sequenced. With BLASTn, CCTCC M206119 strain was determined as *L. crispatus* at the *16S rRNA* gene level (Figure 5D).

DISCUSSION

IBD, including CD and UC, are chronic immune-mediated diseases in which endogenous bacteria are thought to play an important role, as suggested by many clinical observations and experimental studies summarized in recent reviews[1]. Recent studies have also shown that some bacterial strains or mixtures may have the capacity to promote or reduce intestinal inflammation[4].

Although the pathogenesis of IBD remains elusive, the relevance of intestinal luminal bacteria in the initiation and progression of chronic intestinal inflammatory disorders is gaining support[30]. However, no specific microorganism has been associated with the pathogenesis of IBD, suggesting that qualitative/quantitative differences in the intestinal microbiota may play some role in the initiation or perpetuation of intestinal inflammation.

In the current study, we compared the phenotypes of DSS-colitis mice treated by saline, 5-ASA, and CCTCC M206119 strain, trying to determine the role of the probiotic bacterium. We demonstrated that CCTCC M206119 treatment reduced survival rate of DSS-colitis mice. CCTCC-M206119-treated DSS-colitis animals had markedly more severe disease, with greater weight loss, diarrhea, fecal bleeding, and shortened colon length. In addition, the CCTCC-M206119-treated DSS-colitis group had comparatively higher histological scores and more neutrophil infiltration than the controls. To our surprise, CCTCC M206119 strain could be characterized as *L. crispatus* by microscopic morphology, biochemical tests and *16S rRNA* gene level. Hence, our study suggested that not all probiotics are safe for treatment of colitis. Instead, probiotics safety should be carefully evaluated before their application.

Other recent studies also have shown supporting evidence. A strain of *Lactobacillus salivarius* was isolated from blood and bile pus cultures of a 70-year-old man with bacteremic acute cholecystitis[31]. Administration of different lactobacilli and a *Bifidobacterium* strain in an acute liver injury rat model has shown different effects on bacterial translocation and hepatocellular damage. *Bifidobacterium animalis* NM2 increases bacterial translocation to the MLNs but does not affect hepatocellular damage[32]. Together with our data, some probiotics could act as op-

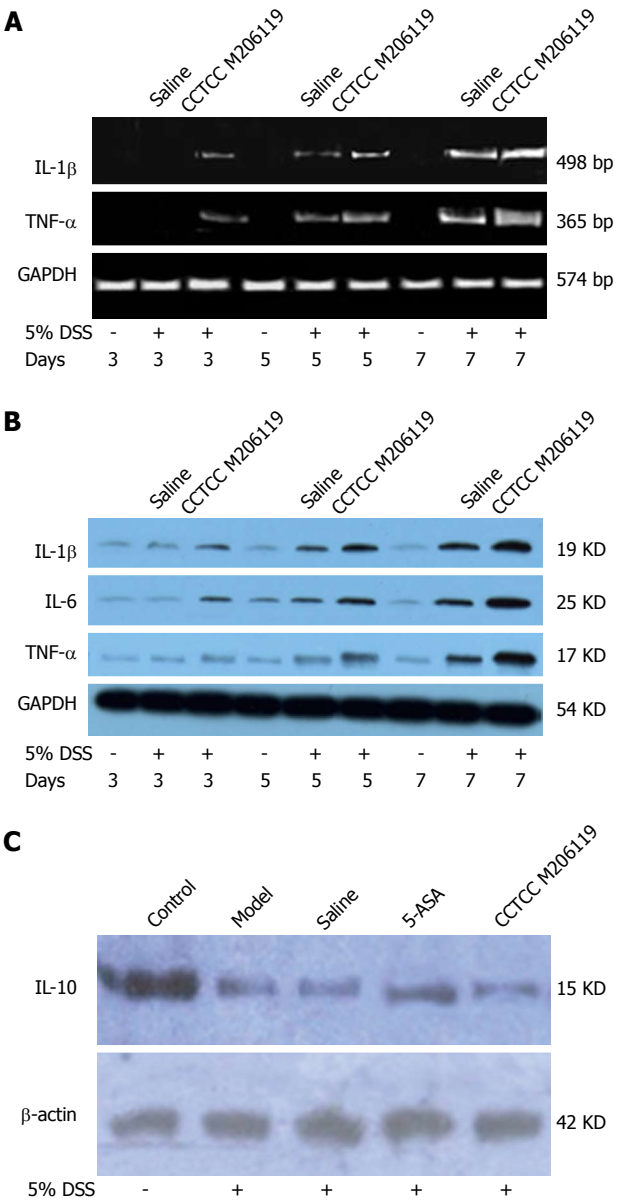
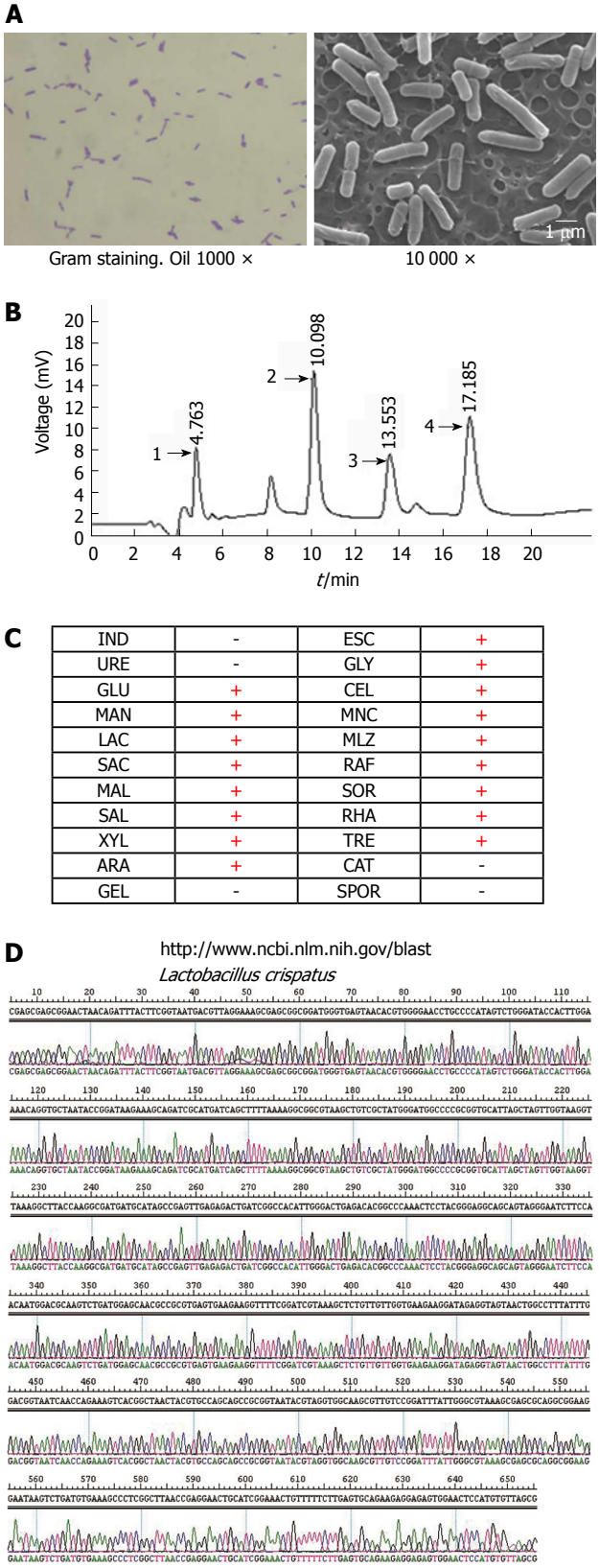


Figure 4 Colonic cytokines changes in China Center for Type Culture Collection M206119 or vehicle-treated dextran sulfate sodium-colitis mice. Expression of interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α from the colons of dextran sulfate sodium (DSS)-colitis mice treated with China Center for Type Culture Collection (CCTCC) M206119 or saline was measured by (A) RT-PCR and (B) western blotting at indicated time points; GAPDH was used as a loading control; C: IL-10 expression from colons of DSS-colitis mice treated with saline, 5-aminosalicylic acid (5-ASA), or CCTCC M206119 was measured by western blotting at indicated time points; β-actin was used as a loading control. The data represent at least three experiments. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

portunistic pathogens and result in endogenous infection unexpectedly. Also, some researchers have demonstrated that the activities to promote endogenous infections are strain specific.

As we know, most lactobacilli have a remarkable record of safety and have been consumed by humans for decades. However, the possible involvement of certain strains has been described in cases of sepsis, endocarditis, or bacteremia; mostly in association with a severe underlying disease



IND	-	ESC	+
URE	-	GLY	+
GLU	+	CEL	+
MAN	+	MNC	+
LAC	+	MLZ	+
SAC	+	RAF	+
MAL	+	SOR	+
SAL	+	RHA	+
XYL	+	TRE	+
ARA	+	CAT	-
GEL	-	SPOR	-

Figure 5 Characteristics of China Center for Type Culture Collection M206119 strain. China Center for Type Culture Collection (CCTCC) M206119 strain was identified by (A) microscopy (Gram staining, oil 1000 ×) and scanning electron microscopy (10 000 ×); (B) Gas chromatography ratio (high performance liquid chromat); (C) biochemical reaction; and (D) sequence analyses of *16S rRNA* gene fragments amplified from DNA eluted from CCTCC M206119 genomic DNA.

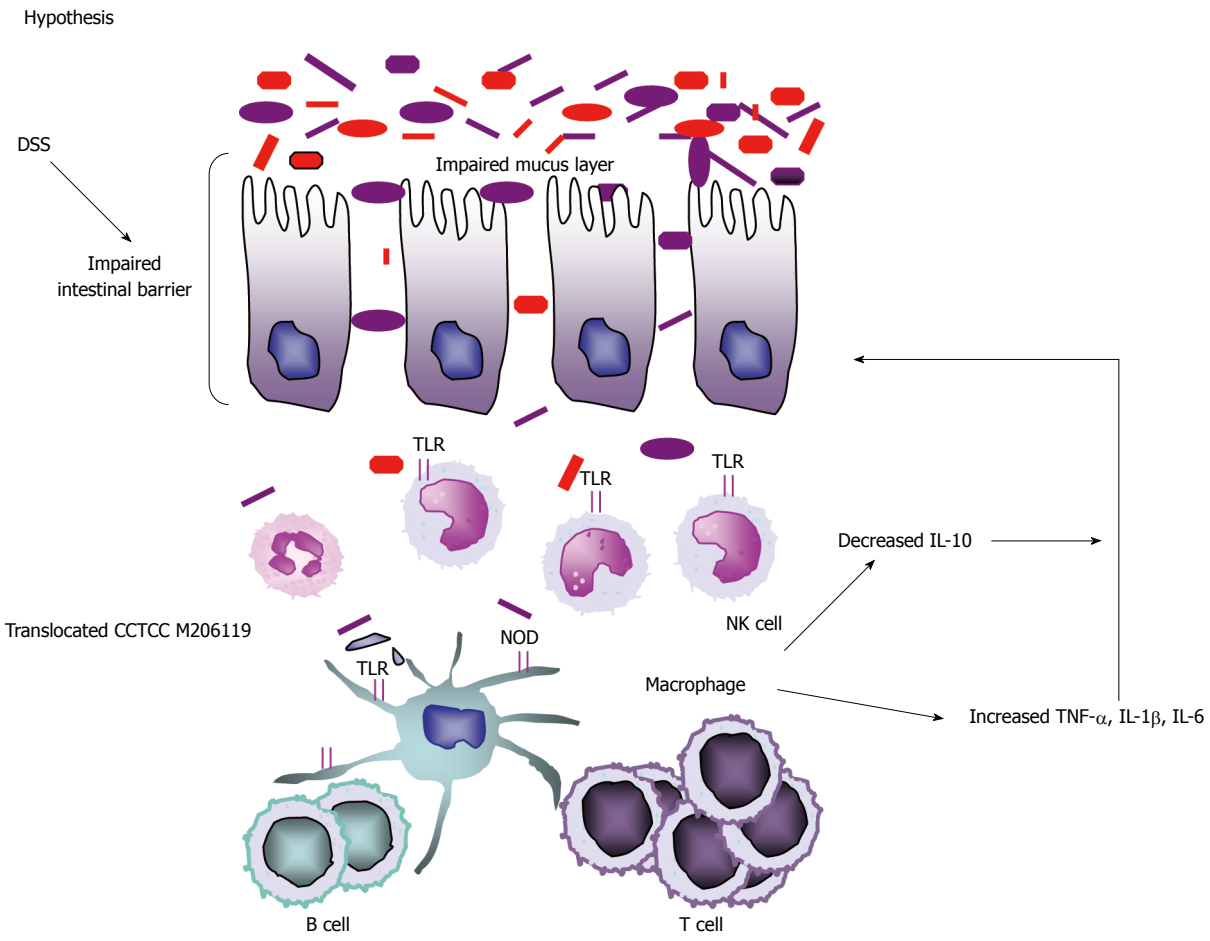


Figure 6 Model illustrating how China Center for Type Culture Collection M206119strain leads to aggravated phenotype in dextran sulfate sodium-colitis mice. DSS: Dextran sulfate sodium; TLR: Toll-like receptor; IL: Interleukin; TNF: Tumor necrosis factor; NOD: Nucleotide-binding oligomerization domain; NK: Natural killer.

or detrimental condition[33]. In view of these facts, the safety of potential probiotic microorganisms in these conditions should be assessed individually. Bacterial translocation (BT) is most likely the first step in the possible passage of viable (indigenous) bacteria to sterile body sites and is thus important for sepsis, endocarditis, and bacteremia caused by the commensal flora. In animal studies, mono association experiments have shown that the type of colitis is dependent on the bacterial species[12,34].

Based on our research, *E. coli* and staphylococci were increased in DSS-colitis mice, whereas lactobacilli in the colon luminal contents were reduced significantly in all DSS-colitis groups, with no significant changes in *Bifidobacterium* or *Enterococcus spp*. Furthermore, our data indicated that changes in gut flora after *L. crispatus* CCTCC M206119 treatment were related to reduced ZO-1 expression and increased β-defensin 2 expression. Our *in vitro* experiment showed that both TNF-α and CCTCC M206119 strain upregulated the nuclear translocation of NF-κB p65, with more positively stained cells in the latter group. Other studies have found that NF-κB can be activated in both intestinal epithelium and intestinal lamina propria mononuclear cells (LPMCs) in acute DSS-colitis mice[35,36]. Intrarectal administration of a*ntisense* oligonucleotides to NF-κB P65 could alleviate the inflammation[24,37]. Thus, it is reasonable to conclude that *L. crispatus* CCTCC M206119 strain exacerbated the imbalance of gut flora; changed the pathogen species or colonies adhered to the colonic mucosa; subsequently altered the expression of tight junction proteins and antibacterial molecules; and activated nuclear translocation of NF-κB p65 in epithelium or inflammatory response cells.

It is thought that IBD might be due to complex mucosal immune responses to antigens of resident enteric bacteria[38-40]. IL-1 and IL-6 have been shown to be the major cytokines secreted by lamina propria cells and play a critical role in the development of Th1-cell-mediated chronic colitis[41]. Recombined IL-1 receptor antagonist can alleviate the mucosal inflammation and necrosis in DSS-coltis mice[42]. DSS-induced colitis was less severe in *IL-6* gene knockout mice, whereas transgenic mice with a mutant *CIS/SOCS3* gene, encoding a negative regulator for the IL-6/STAT3 (signal transducer and activator of transcription) signaling pathway, had increased susceptibility to DSS-induced colitis[43]. In our study, we found that both mRNA and protein levels of IL-1 β, IL-6 and TNF-α from colons of CCTCC-M206119-treated DSS-colitis mice were significantly higher and earlier than those from colons of saline-treated DSS-colitis mice. In contrast, we found that expression of anti-inflammatory

cytokine IL-10 was dramatically downregulated in saline- and CCTCC-M206119-treated DSS-colitis mice, with more significant change in the latter group. This indicated that DSS treatment resulted in increased expression of proinflammatory cytokines and decreased anti-inflammatory cytokines, whereas DSS plus CCTCC M206119 treatment resulted in significantly higher and earlier expression of the proinflammatory cytokines and inhibited the expression of anti-inflammatory cytokine in DSS-colitis mice.

IL-10 can inhibit the expression of IL-1α, IL-1β, IL-6, IL-12, IL-18, granulocyte-macrophage colony-stimulating factor (GM-CSF), G-CSF, M-CSF, TNF, leukemia inhibitory factor and platelet activating factor produced by activated monocytes or macrophages. It exerts strong immune inhibitory function[8] and plays a major role in the immune tolerance of intestinal mucosa[9,10]. Thus, it is possible that administration of *L. crispatus* CCTCC M206119 strain interrupts the balance between anti-inflammatory and proinflammatory responses in colonic mucosa or lamina propria after DSS treatment, and leads to subsequently aggravated colonic inflammation. These processes are similar to those observed in UC patients[11].

Thus, our hypothesis in this study is as follows: some bacteria or bacterial products, such as *L. crispatus* CCTCC M206119, may interact directly with colonic epithelial cells or LPMCs after disruption of the mucosal barrier and balance of gut flora by DSS administration. Then neutrophils and mononuclear cells infiltrate the lamina propria and activate NF-κB translocation, which in turn increases proinflammatory cytokines such as IL-1β, IL-6 and TNF-α. Furthermore, TNF-α can augment NF-κB activation in various cell types, and inhibit the production of anti-inflammatory cytokines such as IL-10 (Figure 6).

Overall, human IBD trials to date have suggested that probiotics display no overt side effects, but conflicting reports on probiotic efficacy highlight the importance of selecting well-characterized probiotic strains and in delivering intact pharmaceutical formulations at an appropriate dose level to the inflamed regions of the intestine. These studies have emphasized that certain probiotic strains need to be thoroughly investigated *in vitro* and *in vivo* using appropriate disease models. Also, some probiotics such as *L. crispatus* CCTCC M206119 might act as aggravating factors in the pathogenesis of UC. Future studies to identify associations between microorganisms (and or their genes and gene products) and human physiological or disease processes will lead to the development and testing of hypotheses that address causality as well as a myriad of specific host-microbe interactions.

ACKNOWLEDGMENTS

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COMMENTS

Background

It is widely accepted that a combination of genetic factors, immune disorders and environmental factors could be involved in the etiology of inflammatory bowel disease (IBD). Recent research has illustrated that some commensal and pathogenic bacteria are closely related to IBD. However, to date, the role of bacteria in the pathogenesis of IBD, or the efficacy of probiotics is still controversial.

Research frontiers

The efficiency of probiotics in controlling IBD is still controversial. Thus, it is difficult to draw a definitive conclusion in evaluating the role of microflora in the pathogenesis of IBD, and subsequently, the efficacy in controlling IBD. Some published data have also questioned the safety of probiotics.

Innovations and breakthroughs

Microflora considered beneficial to the host include the genera *Bifidobacterium* and *Lactobacillus*. In this context, several candidate strains were screened to find some probiotics as therapeutics using dextran sodium sulfate (DSS)-induced colitis in mice, and China Center for Type Culture Collection (CCTCC) M206119 strain led to an exacerbated phenotype of DSS-colitis mice. Expression of protective factors zonula occludens-1 and β-defensin 2 was downregulated after CCTCC M206119 treatment. There was an increase in the nuclear translocation of nuclear factor-κB in epithelial cells. Then, intestinal proinflammatory and anti-inflammatory cytokine responses were evaluated. Proinflammatory colonic cytokines [interleukin (IL)-1β, IL-6 and tumor necrosis factor-α] levels were clearly increased in CCTCC-M206119-treated animals, whereas anti-inflammatory colonic cytokine (IL-10) level was lowered compared with saline- or 5-ASA-treated DSS-colitis mice. Next, CCTCC M206119 strain was identified as *Lactobacillus crispatus*, so it was concluded that not all lactobacilli strains have beneficial effects on intestinal inflammation and that *Lactobacillus crispatus* (*L. crispatus*) CCTCC M206119 is involved in exacerbation of intestinal inflammation in DSS-colitis mice.

Applications

The study results suggest that some probiotics such as *L. crispatus* CCTCC M206119 strain might act as an aggravating factor in the pathogenesis of ulcerative colitis (UC). They also highlight the importance of selecting well-characterized probiotic strains and in delivering intact pharmaceutical formulations at an appropriate dose level to the inflamed regions of the intestine.

Terminology

IBD is a group of inflammatory conditions of the colon and small intestine, including Crohn's disease and UC, which are characterized by abnormal activation of the gut-associated immune system, resulting in chronic inflammation of the digestive tract. *Lactobacillus* is a genus of Gram-positive facultative anaerobic or microaerophilic rod-shaped bacteria. They are a major part of the lactic acid bacteria group, named as such because most of its members convert lactose and other sugars to lactic acid. They are common and usually benign.

Peer review

In this study, the authors investigated the role of CCTCC M206119 strain on intestinal inflammation using a DSS-induced colitis mice model. They showed that CCTCC M206119 strain was identified as *L. crispatus*, but this strain was involved in the exacerbation of intestinal inflammation in DSS-colitis mice. From these results, the authors concluded that not all lactobacilli strains have beneficial effects on intestinal inflammation. This paper has been well written and the results are interesting.

REFERENCES

1 **Xavier RJ**, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007; **448**: 427-434

2 **Strober W**, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. *J Clin Invest* 2007; **117**: 514-521

3 **Heimesaat MM**, Fischer A, Siegmund B, Kupz A, Niebergall J, Fuchs D, Jahn HK, Freudenberg M, Loddenkemper C, Batra A, Lehr HA, Liesenfeld O, Blaut M, Göbel UB, Schumann

RR, Bereswill S. Shift towards pro-inflammatory intestinal bacteria aggravates acute murine colitis via Toll-like receptors 2 and 4. *PLoS One* 2007; **2**: e662
4 **Sartor RB**. Microbial influences in inflammatory bowel diseases. *Gastroenterology* 2008; **134**: 577-594
5 **Hans W**, Schölmerich J, Gross V, Falk W. The role of the resident intestinal flora in acute and chronic dextran sulfate sodium-induced colitis in mice. *Eur J Gastroenterol Hepatol* 2000; **12**: 267-273
6 **Madsen KL**, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 1999; **116**: 1107-1114
7 **Ohkusa T**, Nomura T, Terai T, Miwa H, Kobayashi O, Hojo M, Takei Y, Ogihara T, Hirai S, Okayasu I, Sato N. Effectiveness of antibiotic combination therapy in patients with active ulcerative colitis: a randomized, controlled pilot trial with long-term follow-up. *Scand J Gastroenterol* 2005; **40**: 1334-1342
8 **Kuehbacher T**, Rehman A, Lepage P, Hellmig S, Fölsch UR, Schreiber S, Ott SJ. Intestinal TM7 bacterial phylogenies in active inflammatory bowel disease. *J Med Microbiol* 2008; **57**: 1569-1576
9 **Nishikawa J**, Kudo T, Sakata S, Benno Y, Sugiyama T. Diversity of mucosa-associated microbiota in active and inactive ulcerative colitis. *Scand J Gastroenterol* 2009; **44**: 180-186
10 **Lakatos PL**, Fischer S, Lakatos L, Gal I, Papp J. Current concept on the pathogenesis of inflammatory bowel disease-crosstalk between genetic and microbial factors: pathogenic bacteria and altered bacterial sensing or changes in mucosal integrity take "toll" ? *World J Gastroenterol* 2006; **12**: 1829-1841
11 **Zhang M**, Liu B, Zhang Y, Wei H, Lei Y, Zhao L. Structural shifts of mucosa-associated lactobacilli and Clostridium leptum subgroup in patients with ulcerative colitis. *J Clin Microbiol* 2007; **45**: 496-500
12 **Peña JA**, Li SY, Wilson PH, Thibodeau SA, Szary AJ, Versalovic J. Genotypic and phenotypic studies of murine intestinal lactobacilli: species differences in mice with and without colitis. *Appl Environ Microbiol* 2004; **70**: 558-568
13 **Vanderpool C**, Yan F, Polk DB. Mechanisms of probiotic action: Implications for therapeutic applications in inflammatory bowel diseases. *Inflamm Bowel Dis* 2008; **14**: 1585-1596
14 **Kühbacher T**, Ott SJ, Helwig U, Mimura T, Rizzello F, Kleessen B, Gionchetti P, Blaut M, Campieri M, Fölsch UR, Kamm MA, Schreiber S. Bacterial and fungal microbiota in relation to probiotic therapy (VSL#3) in pouchitis. *Gut* 2006; **55**: 833-841
15 **Mimura T**, Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot IC, Nicholls RJ, Gionchetti P, Campieri M, Kamm MA. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut* 2004; **53**: 108-114
16 **Osman N**, Adawi D, Ahrne S, Jeppsson B, Molin G. Modulation of the effect of dextran sulfate sodium-induced acute colitis by the administration of different probiotic strains of Lactobacillus and Bifidobacterium. *Dig Dis Sci* 2004; **49**: 320-327
17 **Geier MS**, Butler RN, Giffard PM, Howarth GS. Lactobacillus fermentum BR11, a potential new probiotic, alleviates symptoms of colitis induced by dextran sulfate sodium (DSS) in rats. *Int J Food Microbiol* 2007; **114**: 267-274
18 **Rochat T**, Bermúdez-Humarán L, Gratadoux JJ, Fourage C, Hoebler C, Corthier G, Langella P. Anti-inflammatory effects of Lactobacillus casei BL23 producing or not a manganese-dependant catalase on DSS-induced colitis in mice. *Microb Cell Fact* 2007; **6**: 22
19 **Marteau P**, Lémann M, Seksik P, Laharie D, Colombel JF, Bouhnik Y, Cadiot G, Soulé JC, Bourreille A, Metman E, Lerebours E, Carbonnel F, Dupas JL, Veyrac M, Coffin B, Moreau J, Abitbol V, Blum-Sperisen S, Mary JY. Ineffectiveness of Lactobacillus johnsonii LA1 for prophylaxis of postoperative recurrence in Crohn's disease: a randomised, double blind, placebo controlled GETAID trial. *Gut* 2006; **55**: 842-847
20 **Van Gossum A**, Dewit O, Louis E, de Hertogh G, Baert F, Fontaine F, DeVos M, Enslen M, Paintin M, Franchimont D. Multicenter randomized-controlled clinical trial of probiotics (Lactobacillus johnsonii, LA1) on early endoscopic recurrence of Crohn's disease after lleo-caecal resection. *Inflamm Bowel Dis* 2007; **13**: 135-142
21 **Moon G**, Myung SJ, Jeong JY, Yang SK, Cho YK, Lee SM, Chang HS, Byeon JS, Lee YJ, Lee GH, Hong WS, Kim JH, Min YI, Kim JS. [Prophylactic effect of Lactobacillus GG in animal colitis and its effect on cytokine secretion and mucin gene expressions]. *Korean J Gastroenterol* 2004; **43**: 234-245
22 **Lian GH,** Lu FG, Chen HH, You Y, Tan X, Qiu L. Effects of B.adolescentis and L.acidophilus in treating experimental ulcerative colitis in mice and their potential mechanisms. *Zhonghua Xiaohua Zazhi* 2008; **28**: 102-104
23 **Cooper HS**, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest* 1993; **69**: 238-249
24 **Murano M**, Maemura K, Hirata I, Toshina K, Nishikawa T, Hamamoto N, Sasaki S, Saitoh O, Katsu K. Therapeutic effect of intracolonically administered nuclear factor kappa B (p65) antisense oligonucleotide on mouse dextran sulphate sodium (DSS)-induced colitis. *Clin Exp Immunol* 2000; **120**: 51-58
25 **Hamamoto N**, Maemura K, Hirata I, Murano M, Sasaki S, Katsu K. Inhibition of dextran sulphate sodium (DSS)-induced colitis in mice by intracolonically administered antibodies against adhesion molecules (endothelial leucocyte adhesion molecule-1 (ELAM-1) or intercellular adhesion molecule-1 (ICAM-1)). *Clin Exp Immunol* 1999; **117**: 462-468
26 **Dieleman LA**, Palmen MJ, Akol H, Bloemena E, Peña AS, Meuwissen SG, Van Rees EP. Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. *Clin Exp Immunol* 1998; **114**: 385-391
27 **Chiva M**, Soriano G, Rochat I, Peralta C, Rochat F, Llovet T, Mirelis B, Schiffrin EJ, Guarner C, Balanzó J. Effect of Lactobacillus johnsonii La1 and antioxidants on intestinal flora and bacterial translocation in rats with experimental cirrhosis. *J Hepatol* 2002; **37**: 456-462
28 **Brown AC**, Shovic A, Ibrahim SA, Holck P, Huang A. A non-dairy probiotic's (poi) influence on changing the gastrointestinal tract's microflora environment. *Altern Ther Health Med* 2005; **11**: 58-64
29 **Fabia R**, Ar'Rajab A, Johansson ML, Willén R, Andersson R, Molin G, Bengmark S. The effect of exogenous administration of Lactobacillus reuteri R2LC and oat fiber on acetic acid-induced colitis in the rat. *Scand J Gastroenterol* 1993; **28**: 155-162
30 **Agius LM**. A primary dysregulation in the immunoregulatory role of the intestinal mucosal epithelial cell in inflammatory bowel disease pathogenesis? Biology of inflammatory response as tissue pattern entities in Crohn's versus ulcerative colitis. *J Theor Biol* 2004; **227**: 219-228
31 **Woo PC**, Fung AM, Lau SK, Yuen KY. Identification by 16S rRNA gene sequencing of Lactobacillus salivarius bacteremic cholecystitis. *J Clin Microbiol* 2002; **40**: 265-267
32 **Adawi D**, Ahrné S, Molin G. Effects of different probiotic strains of Lactobacillus and Bifidobacterium on bacterial translocation and liver injury in an acute liver injury model. *Int J Food Microbiol* 2001; **70**: 213-220
33 **Daniel C**, Poiret S, Goudercourt D, Dennin V, Leyer G, Pot B. Selecting lactic acid bacteria for their safety and functionality by use of a mouse colitis model. *Appl Environ Microbiol* 2006; **72**: 5799-5805

34 **Foligne B**, Dessein R, Marceau M, Poiret S, Chamaillard M, Pot B, Simonet M, Daniel C. Prevention and treatment of colitis with Lactococcus lactis secreting the immunomodulatory Yersinia LcrV protein. *Gastroenterology* 2007; **133**: 862-874
35 **Tessner TG**, Cohn SM, Schloemann S, Stenson WF. Prostaglandins prevent decreased epithelial cell proliferation associated with dextran sodium sulfate injury in mice. *Gastroenterology* 1998; **115**: 874-882
36 **Sakuraba H**, Ishiguro Y, Yamagata K, Tagawa Y, Iwakura Y, Sekikawa K, Munakata A, Nakane A. Transforming growth factor-{beta} regulates susceptibility of epithelial apoptosis in murine model of colitis. *Ann N Y Acad Sci* 2004; **1029**: 382-384
37 **Dijkstra G**, Moshage H, Jansen PL. Blockade of NF-kappaB activation and donation of nitric oxide: new treatment options in inflammatory bowel disease? *Scand J Gastroenterol Suppl* 2002; 37-41
38 **Sartor RB**. Probiotic therapy of intestinal inflammation and infections. *Curr Opin Gastroenterol* 2005; **21**: 44-50
39 **Sartor RB**. Targeting enteric bacteria in treatment of inflammatory bowel diseases: why, how, and when. *Curr Opin Gastroenterol* 2003; **19**: 358-365
40 **Sartor RB**. Induction of mucosal immune responses by bacteria and bacterial components. *Curr Opin Gastroenterol* 2001; **17**: 555-561
41 **Yamamoto M**, Yoshizaki K, Kishimoto T, Ito H. IL-6 is required for the development of Th1 cell-mediated murine colitis. *J Immunol* 2000; **164**: 4878-4882
42 **Peterson RL**, Wang L, Albert L, Keith JC, Dorner AJ. Molecular effects of recombinant human interleukin-11 in the HLA-B27 rat model of inflammatory bowel disease. *Lab Invest* 1998; **78**: 1503-1512
43 **Matsumoto S**, Hara T, Hori T, Mitsuyama K, Nagaoka M, Tomiyasu N, Suzuki A, Sata M. Probiotic Lactobacillus-induced improvement in murine chronic inflammatory bowel disease is associated with the down-regulation of pro-inflammatory cytokines in lamina propria mononuclear cells. *Clin Exp Immunol* 2005; **140**: 417-426

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BRIEF ARTICLE

Endoscopic ultrasonography-guided fine needle aspiration: Relatively low sensitivity in the endosonographer population

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Abstract

AIM: To assess the characteristics and quality of endoscopic ultrasonography-guided fine needle aspiration (EUS-FNA) in a large panel of endosonographers.

METHODS: A survey was conducted during the 13th annual live course of endoscopic ultrasonography (EUS) held in Amsterdam, Netherlands. A 2-page questionnaire was developed for the study. Content validity of the questionnaire was determined based on input by experts in the field and a review of the relevant literature. It contained 30 questions that pertained to demographics and the current practice for EUS-FNA of responders, including sampling technique, sample processing, cytopathological diagnosis and sensitivity of EUS-FNA for the diagnosis of solid mass lesions. One hundred and sixty-one endosonographers who attended the course were asked to answer the survey. This allowed assessing the current practice of EUS-FNA as well as the self-reported sensitivity of EUS-FNA for the diagnosis of solid mass lesions. We also examined which factors were associated with a self-reported sensitivity of EUS-FNA for the diagnosis of solid mass lesions > 80%.

RESULTS: Completed surveys were collected from 92 (57.1%) of 161 endosonographers who attended the conference. The endosonographers had been practicing endoscopy and EUS for 12.5 ± 7.8 years and 4.8 ± 4.1 years, respectively; one third of them worked in a hospital with an annual caseload > 100 EUS-FNA. Endoscopy practices were located in 29 countries, including 13 countries in Western Europe that totaled 75.3% of the responses. Only one third of endosonographers reported a sensitivity for the diagnosis of solid mass lesions > 80% (interquartile range of sensitivities, 25.0%-75.0%). Factors independently associated with a sensitivity > 80% were (1) > 7 needle passes for pancreatic lesions or rapid on-site cytopathological evaluation (ROSE) (P < 0.0001), (2) a high annual hospital caseload (P = 0.024) and (3) routine isolation of microcores from EUS-FNA samples (P = 0.042). ROSE was routinely available to 27.9% of respondents. For lymph nodes and pancreatic masses, a maximum of three needle passes was performed by approximately two thirds of those who did not have ROSE. Microcores were routinely harvested from EUS-FNA samples by approximately one third (37.2%) of survey respondents.

CONCLUSION: EUS-FNA sensitivity was considerably lower than reported in the literature. Low EUS-FNA sensitivity was associated with unavailability of ROSE, few needle passes, absence of microcore isolation and low hospital caseload.

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Key words: Caseload; Community surveys; Cytopa-

thology; Endoscopic ultrasonography; Histopathology; Quality improvement

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INTRODUCTION

Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) has become widely available in a variety of endoscopy settings. Due to its high diagnostic accuracy, EUS-FNA plays an important role in the management of masses located in or close to the gastrointestinal tract. For example, median sensitivity of EUS-FNA to differentiate between benign and malignant masses of the pancreas (technically the most difficult location)[1], is reported to be greater than 80%[2]. Such a high sensitivity for malignancy diagnosis has allowed EUS-FNA to significantly impact patient management in various clinical situations, including pancreatic cancer[3], mediastinal lesions[4,5], lung cancer[6], and solid liver lesions[7,8].

Excellent results of EUS-FNA such as those cited above have mostly been reported by dedicated endoscopists, many of them working in academic centers. The quality of EUS-FNA in the endosonographer population is poorly known, despite the fact that the quality of endoscopic procedures has become a central concern for the American and European Societies of Gastrointestinal Endoscopy[9-11].

To characterize the situation of EUS-FNA in the community, we performed a survey in a large audience of endosonographers to assess (1) current modalities of EUS-FNA; (2) self-reported sensitivity of EUS-FNA for the diagnosis of solid masses; and (3) factors associated with a diagnostic yield similar to that reported in the literature.

MATERIALS AND METHODS

Survey design and administration

A 2-page, 30-item, questionnaire (Appendix 1) was developed for the study. Content validity of the survey was determined based on input by experts in the field and a review of the relevant literature. Participants were asked to answer questions pertaining to demographics (6 items), their own current practice for EUS-FNA (16 items), sample processing (3 items), cytopathological diagnosis (3 items) and sensitivity of EUS-FNA for the diagnosis of solid mass lesions.

The survey was conducted during the 13th annual live EUS course held in Amsterdam, Netherlands, June 3rd and 4th 2010, as previously described[12,13]. Briefly, questionnaires were placed in bags distributed to course participants, and attendees were asked to deposit completed surveys in a dedicated box at the registration desk. Consent to participate in this study was inferred from voluntary completion of the survey. Efforts to increase response rates included reminders by the course director and moderators, projection of a reminder slide during breaks, and collection of surveys by staff members. No gift or financial incentive was granted to attendees.

Statistical analysis

Results are expressed as mean ± SD or as a percentage. Answers for each individual question were obtained from all survey respondents except when otherwise stated (each response was included in the analysis, regardless of the completeness of the survey); therefore, the number of respondents for each individual question (i.e., the denominator for percentage calculations) is indicated.

Comparisons between groups were performed with the Pearson χ^2 test or Fisher's exact test (Freeman-Halton extension where applicable) for categorical data and the Wilcoxon signed rank test for continuous variables. We also examined, by using multiple logistic regression analysis, which factors-including years of EUS practice, EUS-FNA annual hospital caseload, availability of rapid on-site cytopathological evaluation (ROSE) of EUS-FNA samples, number of needle passes > 7 or based on ROSE for three lesion types (lymph node, pancreas mass < 25 mm, pancreas mass > 25 mm), method of EUS-FNA sample preservation and routine isolation of microcores from EUS-FNA samples-were associated with a sensitivity of EUS-FNA for the diagnosis of solid mass lesions > 80%. All tests were 2-sided and P values < 0.05 were considered statistically significant. All analyses were performed with JMP software (version 9.0.0; SAS, Cary, NC, United States).

RESULTS

Study population

Completed surveys were collected from 92 (57.1%) of 161 endosonographers who attended the conference (excluding international faculty members, local faculty, fellows, and industry delegates). Survey respondents had been practicing endoscopy and EUS for 12.5 ± 7.8 years and 4.8 ± 4.1 years, respectively; 38 (42%) of them had been performing EUS for more than 5 years (Table 1). Approximately one third of respondents were practicing in a center with an annual caseload > 100 EUS-FNA. Endoscopy practices were located in 29 countries, including 13 countries in Western Europe (Austria, Belgium, Denmark, Germany, France, Greece, Italy, Netherland, Portugal, Spain, Sweden, Switzerland and the United Kingdom) that totaled 75.3% (67 out of 89) of the responses. Most (77.1%) survey respondents were performing FNA or drainage in fewer than 40% of EUS procedures. For patients subjected to both endoscopic biliary drainage

Table 1 Characteristics of survey respondents *n* (%)

Male gender (Nr = 92)	66 (71.7)
Age (yr) (Nr = 92)	43.1 ± 8.0
Years of endoscopy practice (Nr = 92)	12.5 ± 7.8
Years of EUS practice (Nr = 91)	4.8 ± 4.1
Number of respondents practicing in Western Europe (Nr = 89)	67 (75.3)
Proportion of EUS with FNA or drainage (Nr = 92)	
< 20%	35 (38)
20%-40%	36 (39.1)
40%-60%	18 (19.6)
60%-80%	3 (3.3)
> 80%	0
EUS-FNA annual hospital caseload (Nr = 92)	
< 50	32 (34.8)
50-100	26 (28.3)
100-200	25 (27.2)
> 200	9 (9.8)
Sensitivity of EUS-FNA for the diagnosis of solid mass lesions (Nr = 61)	
< 40%	1 (1.6)
40%-60%	14 (23.0)
60%-80%	23 (37.7)
> 80%	23 (37.7)

Numbers are means ± SD except where stated otherwise. N_r: Number of respondents for each question; ERCP: Endoscopic retrograde cholangiopancreatography; EUS: Endoscopic ultrasonography; EUS-FNA: Endoscopic ultrasonography-guided fine needle aspiration; FNA: Fine needle aspiration.

and EUS-FNA, only 27% of survey respondents were performing both procedures during a single endoscopy session. Staging of pulmonary cancer was performed by approximately one third of survey respondents (27 of 86; 31.4%).

Practice of EUS-FNA

A 22G needle was used for all lesion types by the majority of endosonographers; a 25G needle was used by 19.8% of survey respondents for EUS-FNA of the pancreas head (as compared to 6.8% for esophagogastric lesions; $P = 0.009$) (Table 2). ROSE of EUS-FNA samples was available to less than half of survey respondents, routinely (27.9% of answers) or in selected cases (15.1% of answers). Most endosonographers who had ROSE routinely available used it to determine the number of needle passes during EUS-FNA, while a maximum of three needle passes was performed by approximately two thirds of those who did not have ROSE routinely available. Samples were prepared using liquid-based methods plus smearing by approximately half of survey respondents (46.5%); microcores were routinely harvested from EUS-FNA samples by approximately one third (37.2%) of survey respondents. Paraffin-embedded blocks were prepared for histopathological examination of EUS-FNA samples in the laboratory of approximately half of survey respondents (55.8%). Forty-five (52.9%) of 85 survey respondents had regular meetings with the pathologist examining EUS-FNA samples.

If EUS-FNA was repeated after a first inconclusive procedure, survey respondents repeated an identical procedure or referred the patient to another endosonographer in 35.3% and 8.2% of cases, respectively. Those who modified the procedure performed more needle passes, used a larger needle and, in 25% of cases, added ROSE of the EUS-FNA sample. The vast majority of survey respondents performed Doppler examination before EUS-FNA (79 of 88 responses; 89.7%) and administered antibiotic prophylaxis before EUS-FNA of pancreatic fluid collections (82 of 86 responses; 95.3%); antibiotic prophylaxis was less consistently administered in other indications of EUS-FNA (perirectal lesions, 43 (58.1%) of 74 responses; pancreas solid lesions, 10 (11.6%) of 86 responses). For sampling of pancreatic cysts, EUS-guided cyst wall brushing was rarely used (5 of 86 responses, 5.8%).

Table 2 Practice of endoscopic ultrasonography-guided fine needle aspiration *n* (%)

Diameter of the needle used for lesions located	
In the esophagus/stomach (Nr = 88)	
19G	12 (13.6)
22G	70 (79.5)
25G	6 (6.8)
In the head of the pancreas (Nr = 86)	
19G	5 (5.8)
22G	64 (74.4)
25G	17 (19.8)
ROSE available (Nr = 86)	
Routinely	24 (27.9)
In selected cases	13 (15.1)
Never	49 (56.9)
Number of needle passes	
Pancreatic mass < 25 mm (Nr = 84)	
⩽ 3	41 (48.8)
5-7	19 (22.6)
> 7 or based on ROSE	24 (28.6)
Pancreatic mass > 25 mm (Nr = 83)	
⩽ 3	37 (44.6)
5-7	21 (25.3)
> 7 or based on ROSE	25 (30.1)
Lymphadenopathy (Nr = 87)	
⩽ 3	51 (58.6)
5-7	14 (16.1)
> 7 or based on ROSE	22 (25.3)
Paraffin-embedded blocks prepared for histopathological analysis (Nr = 86)	
Yes	48 (55.8)
No	12 (13.9)
Do not know	26 (30.2)
Pathologist making routine diagnosis for EUS-FNA samples (Nr = 85)	
Dedicated digestive cytopathologist	25 (29.4)
General cytopathologist	47 (55.3)
Digestive pathologist not specialized in cytology	13 (15.3)
Attitude if EUS-FNA is repeated after a first inconclusive EUS-FNA (Nr = 85)	
Change in the procedure (2 answers allowed)	48 (56.5)
Higher number of needle passes	42 (87.5)
Larger needle	19 (39.6)
Addition of ROSE	12 (25.0)
Tru-Cut needle in the esophagus and rectum	7 (14.6)
Repetition of identical procedure	30 (35.3)
Referral to another endosonographer	7 (8.2)

N_r: Number of respondents for each question; EUS-FNA: Endoscopic ultrasonography-guided fine needle aspiration; G: Gauge; ROSE: Rapid on-site cytopathological evaluation.

Table 3 Univariate analysis of variables potentially associated with an endoscopic ultrasonography-guided fine needle aspiration sensitivity > 80% for the diagnosis of solid mass lesions

	P value
Number of needle passes based on ROSE or > 7 (small pancreatic lesions)	< 0.0001
Number of needle passes based on ROSE or > 7 (large pancreatic lesions)	0.0002
ROSE available	0.0017
Number of needle passes based on ROSE or > 7 (lymphadenopathy)	0.0019
High annual hospital caseload	0.0242
Routine isolation of microcores	0.0422
Method of sample preservation	0.1198
Years of EUS practice	0.6475

EUS: Endoscopic ultrasonography; ROSE: Rapid on-site cytopathological evaluation.

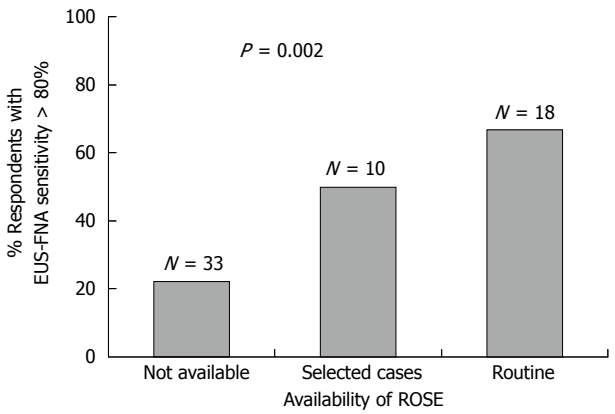

Figure 1 Percentages of respondents with sensitivity > 80% according to availability of rapid on-site cytopathology evaluation (rapid on-site cytopathological evaluation) of endoscopic ultrasonography-guided fine needle aspiration samples. The number above each bar represents the total number of respondents in each category. For example, among 10 respondents who had ROSE available in selected cases, five (50%) self-reported a sensitivity > 80% for the diagnosis of solid mass lesions. EUS-FNA: Endoscopic ultrasonography-guided fine needle aspiration; ROSE: Rapid on-site cytopathological evaluation.

Sensitivity of EUS-FNA

Only one third of endosonographers (37.7%) reported a sensitivity > 80% for the diagnosis of solid mass lesions; the interquartile range of self-reported sensitivities (that represents 50% of the answers, centered on the median value) was 25.0%-75.0%. Factors associated in univariate analysis with a sensitivity greater than 80% are shown in Table 3 and specific associations (availability of ROSE and annual hospital caseload of EUS-FNA) are illustrated in Figures 1 and 2. In multivariate analysis, the only independent factors associated with a sensitivity greater than 80% were (1) the usage of ROSE to determine the number of needle passes or, if no ROSE was available, > 7 passes; (2) a high annual hospital caseload of EUS-FNA; and (3) routine isolation of microcores from EUS-FNA samples. The other associations listed in Table 3 became non-significant after including the number of needle passes for small pancreatic lesions based on ROSE or > 7 passes in the stepwise analysis.

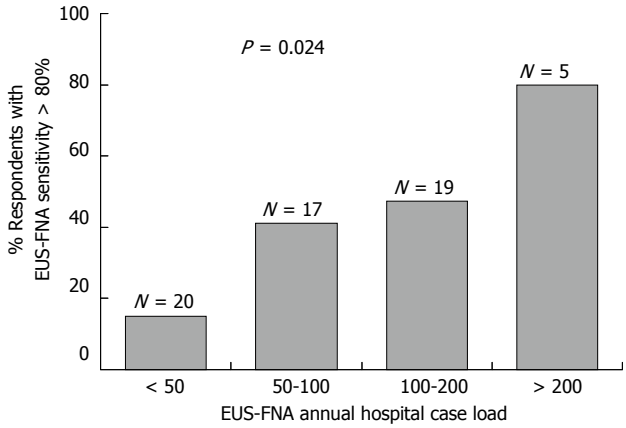

Figure 2 Percentages of respondents with sensitivity > 80% according to annual hospital caseload. The number above each bar represents the total number of respondents in each category. For example, among 20 respondents with an annual hospital caseload < 50 EUS-FNA, three (15.0%) self-reported a sensitivity > 80% for the diagnosis of solid mass lesions. EUS-FNA: Endoscopic ultrasonography-guided fine needle aspiration.

DISCUSSION

The main finding of our survey is that the sensitivity of EUS-FNA for the diagnosis of solid mass lesions was strikingly lower than that reported in the literature. Only one third of endosonographers reported a sensitivity > 80% and one fourth of them reported a sensitivity < 60%. For comparison, a median sensitivity of 83% was reported in a large review of 28 studies that reported the performance of EUS-FNA to differentiate benign *vs* malignant pancreatic masses, technically the most difficult location; only one of these 28 studies reported a sensitivity < 60%[1,2]. The relatively poor performance of EUS-FNA reported by endosonographers in this survey is unlikely to be due to reporting bias as respondents would likely have over-, not under-, estimated their sensitivity for the diagnosis of solid mass lesions.

Quality has become a central concern in endoscopy. The large variability in sensitivities reported by endosonographers (interquartile range of 25%-75%) suggests that there is much more room for quality improvement in EUS-FNA compared to other procedures that have long been scrutinized such as colonoscopy and endoscopic retrograde cholangio-pancreatography (ERCP). For comparison, (1) in the case of ERCP for instance, deep cannulation of the desired duct was validated as a measure of quality; it was achieved in 83.6% of 3210 patients undergoing their first ever ERCP in an audit of 76 endoscopy units[14]; and (2) regarding colonoscopy, cecal intubation rates ranged between 88% and 97% for 9 of 10 endoscopists investigated over a 6-year period[15]. No quality indicator has been recommended by the Societies of Gastrointestinal Endoscopy to assess the diagnostic accuracy of EUS-FNA[16]. Recently, the overall yield of malignancy for pancreatic masses was proposed as a benchmark for EUS-FNA[17].

Factors independently associated with a high sensitivity of EUS-FNA in our study included (1) the usage of ROSE to determine the number of needle passes or, if no ROSE was available, > 7 passes; (2) a high annual hospital caseload of EUS-FNA; and (3) routine isolation of microcores from EUS-FNA samples. The number of needle passes reported by approximately half of endosonographers was ≤ 3 for lymph nodes as well as for pancreatic masses, although a minimum of 5 or even 7 passes have been recommended for the pancreas when ROSE is not available (for lymph nodes, three needle passes are sufficient)[18-20]. In a study of pancreas EUS-FNA, sensitivity for malignant diagnoses increased from 16.7% with one needle pass to 86.7% if more than 7 passes were performed and another study showed that tumor differentiation was the single factor associated with the number of needle passes required to make a diagnosis[18,19]. The low number of needle passes performed by many endosonographers in the pancreas could be related to procedure duration (approximately five minutes are required per needle pass). To shorten procedure duration, Möller *et al*[21] have proposed to perform only two needle passes for solid pancreatic masses, to harvest microcores for histopathological examination and to subject the residual sample to cytopathological examination. Using this technique, they obtained a sensitivity of 82.9% in a multicenter retrospective study that included 192 patients. A new EUS-FNA needle could also facilitate acquisition of samples adequate for histopathological evaluation[22].

Of note, routine isolation of microcores was another independent factor associated with a high sensitivity in our survey. Microcores adequate for histopathological evaluation can be obtained in 83.9%-90.9% of EUS-FNA of pancreatic masses performed using a standard 22G needle (the model used by most of our survey respondents)[21,23,24]. Several nonrandomized studies have suggested that microcores are useful: (1) in the study by Möller *et al*[21], combined cytopathological and histopathological examination was more sensitive than cytopathological or histopathological examination alone for discriminating malignant *vs* benign pancreatic lesions using two needle passes (82.9% *vs* 68.1% *vs* 60.0%, respectively; $P < 0.01$); and (2) in a prospective series of 50 patients with lymphoma, a diagnostic accuracy of 96% was reached by examining microcores obtained using a 19G needle as compared with 57% in another series when cytopathological examination alone was considered[25,26]. Paraffin-embedded cell blocks might represent an alternative to microcore isolation, however, cell blocks are made in the laboratory, not in the endoscopy room, and thus out of the control of the endosonographer (one third of survey respondents did not know if cell blocks were made with the samples that they provided to the laboratory); furthermore cell blocks have not been as well studied as microcores.

ROSE was routinely available to 28% of endosonographers only, compared to 90% in a study performed among 21 centers in the United States, of which 81% were academic[17]. Anecdotal evidence suggests that low ROSE availability in EUS-FNA facilities may be related to logistical issues, lack of perceived benefit and cost even though ROSE has been shown to be cost-effective during EUS- and percutaneous-guided FNA in some settings[27-29]. Studies about the usefulness of ROSE to reach a high diagnostic yield are contradictory. A retrospective comparison of EUS-FNA performed by a single endosonographer in two university hospitals, one with ROSE available and the other without ROSE, is traditionally cited to support the usefulness of ROSE, but differences in patient populations and indications for EUS-FNA between the two hospitals preclude definitive conclusion[30]. In another, more recent, retrospective study of EUS-FNA for pancreatic masses with ROSE available for 43.8% of 520 procedures, ROSE was associated with a higher diagnostic yield in multivariate analysis (odds ratio, 3.1; $P = 0.0001$)[31]. Nevertheless, in a multicenter prospective study that evaluated 409 patients with 474 lesions[32], a similarly high diagnostic accuracy was achieved in centers regardless of ROSE availability and, in a prospective series of 108 consecutive EUS-FNAs performed without ROSE by a single endosonographer[29], diagnostic accuracy of EUS-FNA for pancreatic lesions was 97%. The authors of the latest study attributed their results to high caseload, dedicated endosonography and cytopathology. Our finding that a high sensitivity of EUS-FNA was independently associated with ROSE and the annual hospital caseload supports that view.

A significant relationship between hospital caseload and quality has been demonstrated for few endoscopy procedures. For ERCP, a large administrative study showed that procedural failure rates were lower for inpatients undergoing ERCP at high- compared to low-volume US hospitals but the difference, albeit statistically significant because of the large sample size (> 2500 hospitals), was small (4.7% *vs* 6.0% in hospitals with an annual caseload ≥ 300 *vs* < 50 procedures, respectively)[33]. The difference in sensitivities reported by endosonographers in our study was much larger, with 15.0% *vs* 80.0% of endosonographers reporting a sensitivity greater than 80% depending if they worked in a hospital with an annual caseload < 50 *vs* > 200 EUS-FNAs, respectively ($P = 0.024$), as illustrated in Figure 2. This supports the view that centralization should be discussed for at least some of the EUS-FNA procedures, as has recently been proposed for ERCP[34]. Such a change would be important as only 8.2% of our survey respondents were referring patients to another endosonographer after a first inconclusive EUS-FNA.

Limitations of our survey include selection and recall bias: (1) surveyed endosonographers were working mostly in Western Europe and our findings may not apply to other locations; and (2) self-reported data are exposed to recall and goodwill biases because surveyed professionals tend to give the expected answer more than the real one, which indeed strengthens our main conclusion that the performance of EUS-FNA is significantly lower than

assumed from published studies. In particular, sensitivity for the diagnosis of solid mass lesions was reported by 61 (37.8%) of 161 participants in the EUS course. Because of these limitations (recruitment of participants from a course, response rate), our conclusion that sensitivity for the diagnosis of solid mass lesions is strikingly lower in the community than reported in the literature should be confirmed by larger studies.

In conclusion, the quality of EUS-FNA, as assessed in this survey by its most crucial result, i.e., sensitivity for cancer diagnosis, was relatively low. Low quality was associated with unavailability of ROSE, low number of needle passes for pancreatic EUS-FNA, absence of routine microcore isolation and low annual hospital caseload. Efforts should be made to improve the quality of EUS-FNA amongst endosonographers; we suggest that endoscopists review their sensitivity for cancer diagnosis and, if this is < 80%, then they should modify their practice taking into account the factors cited above. These recommendations may be particularly valuable for endoscopists working in hospitals with a low annual caseload of EUS-FNA.

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COMMENTS

Background

Endoscopic ultrasonography-guided fine needle aspiration (EUS-FNA) has become widely available and is known for its high diagnostic accuracy in academic centers.

Research frontiers

The quality of several endoscopic procedures (e.g., colonoscopy) has become an area of active improvement. However, for EUS-FNA, simple quality parameters (e.g., diagnostic accuracy) are poorly known in the community. Recently, the overall yield of malignancy for pancreatic masses was proposed as a benchmark for EUS-FNA. This parameter is relatively easy to collect but it does not take into account the prevalence of malignant diseases among pancreatic masses subjected to EUS-FNA by a particular endosonographer.

Innovations and breakthroughs

The authors performed a survey of the current practice of EUS-FNA. The self-reported sensitivity of EUS-FNA for the diagnosis of solid mass lesions was considerably lower than commonly reported in the literature. A high sensitivity for the diagnosis of solid mass lesions was associated with the availability of rapid on-site cytopathological evaluation (ROSE), a high number of needle passes, microcore isolation and a high hospital caseload. The data suggest that there is ample room for improvement of the quality of EUS-FNA and provides several clues for improvement.

Applications

Scientific societies play an important role in helping to improve the quality of endoscopic procedures; particular attention in that field could be paid to EUS-FNA. Centralization of some of the EUS-FNA procedures could be explored as a means of achieving that aim.

Terminology

ROSE of material sampled by EUS-FNA is performed by a cytopathologist or a cytotechnician; preliminary diagnoses resulting from ROSE are highly reliable. ROSE allows a reduction in the number of needle passes but its cost-efficacy is debated. Microcore isolation consists of examining the sample obtained by EUS-FNA for the presence of small tissue core samples, transferring them into a preservative (e.g., formalin) for histopathological examination and sending the rest of the material collected for cytopathological analysis.

Peer review

This is an interesting paper on a survey study performed during the 13th annual live EUS course in Amsterdam in June 2010.

REFERENCES

1 **Eisen GM**, Dominitz JA, Faigel DO, Goldstein JA, Petersen BT, Raddawi HM, Ryan ME, Vargo JJ, Young HS, Wheeler-Harbaugh J, Hawes RH, Brugge WR, Carrougher JG, Chak A, Faigel DO, Kochman ML, Savides TJ, Wallace MB, Wiersema MJ, Erickson RA. Guidelines for credentialing and granting privileges for endoscopic ultrasound. *Gastrointest Endosc* 2001; **54**: 811-814

2 **Hartwig W**, Schneider L, Diener MK, Bergmann F, Büchler MW, Werner J. Preoperative tissue diagnosis for tumours of the pancreas. *Br J Surg* 2009; **96**: 5-20

3 **DeWitt J**, Yu M, Al-Haddad MA, Sherman S, McHenry L, Leblanc JK. Survival in patients with pancreatic cancer after the diagnosis of malignant ascites or liver metastases by EUS-FNA. *Gastrointest Endosc* 2010; **71**: 260-265

4 **Savides TJ**, Perricone A. Impact of EUS-guided FNA of enlarged mediastinal lymph nodes on subsequent thoracic surgery rates. *Gastrointest Endosc* 2004; **60**: 340-346

5 **Larsen SS**, Krasnik M, Vilmann P, Jacobsen GK, Pedersen JH, Faurschou P, Folke K. Endoscopic ultrasound guided biopsy of mediastinal lesions has a major impact on patient management. *Thorax* 2002; **57**: 98-103

6 **Bodtger U**, Vilmann P, Clementsen P, Galvis E, Bach K, Skov BG. Clinical impact of endoscopic ultrasound-fine needle aspiration of left adrenal masses in established or suspected lung cancer. *J Thorac Oncol* 2009; **4**: 1485-1489

7 **DeWitt J**, LeBlanc J, McHenry L, Ciaccia D, Imperiale T, Chappo J, Cramer H, McGreevy K, Chriswell M, Sherman S. Endoscopic ultrasound-guided fine needle aspiration cytology of solid liver lesions: a large single-center experience. *Am J Gastroenterol* 2003; **98**: 1976-1981

8 **tenBerge J**, Hoffman BJ, Hawes RH, Van Enckevort C, Giovannini M, Erickson RA, Catalano MF, Fogel R, Mallery S, Faigel DO, Ferrari AP, Waxman I, Palazzo L, Ben-Menachem T, Jowell PS, McGrath KM, Kowalski TE, Nguyen CC, Wassef WY, Yamao K, Chak A, Greenwald BD, Woodward TA, Vilmann P, Sabbagh L, Wallace MB. EUS-guided fine needle aspiration of the liver: indications, yield, and safety based on an international survey of 167 cases. *Gastrointest Endosc* 2002; **55**: 859-862

9 **Bjorkman DJ**, Popp JW. Measuring the quality of endoscopy. *Gastrointest Endosc* 2006; **63**: S1-S2

10 **Polkowski M**, Larghi A, Weynand B, Boustière C, Giovannini M, Pujol B, Dumonceau JM. Learning, techniques, and complications of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Technical Guideline. *Endoscopy* 2012; **44**: 190-206

11 **Dumonceau JM**, Polkowski M, Larghi A, Vilmann P, Giovannini M, Frossard JL, Heresbach D, Pujol B, Fernández-Esparrach G, Vazquez-Sequeiros E, Ginès A. Indications, results, and clinical impact of endoscopic ultrasound (EUS)-guided sampling in gastroenterology: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2011; **43**: 897-912

12 **Dumonceau JM**, Dumortier J, Deviere J, Kahaleh M, Ponchon T, Maffei M, Costamagna G. Transnasal OGD: practice survey and impact of a live video retransmission. *Dig Liver Dis* 2008; **40**: 776-783

13 **Dumonceau JM**, Rigaux J, Kahaleh M, Gomez CM, Vandermeeren A, Devière J. Prophylaxis of post-ERCP pancreatitis: a practice survey. *Gastrointest Endosc* 2010; **71**: 934-939

14 **Williams EJ**, Taylor S, Fairclough P, Hamlyn A, Logan RF, Martin D, Riley SA, Veitch P, Wilkinson M, Williamson PJ, Lombard M. Are we meeting the standards set for endoscopy? Results of a large-scale prospective survey of endoscopic retrograde cholangio-pancreatograph practice. *Gut* 2007; **56**: 821-829

15 **Aslinia F**, Uradomo L, Steele A, Greenwald BD, Raufman JP. Quality assessment of colonoscopic cecal intubation: an analysis of 6 years of continuous practice at a university hospital. *Am J Gastroenterol* 2006; **101**: 721-731

16 **Faigel DO**. Quality, competency and endosonography. *Endoscopy* 2006; **38** Suppl 1: S65-S69

17 **Savides TJ**, Donohue M, Hunt G, Al-Haddad M, Aslanian H, Ben-Menachem T, Chen VK, Coyle W, Deutsch J, DeWitt J, Dhawan M, Eckardt A, Eloubeidi M, Esker A, Gordon SR, Gress F, Ikenberry S, Joyce AM, Klapman J, Lo S, Maluf-Filho F, Nickl N, Singh V, Wills J, Behling C. EUS-guided FNA diagnostic yield of malignancy in solid pancreatic masses: a benchmark for quality performance measurement. *Gastrointest Endosc* 2007; **66**: 277-282

18 **Erickson RA**, Sayage-Rabie L, Beissner RS. Factors predicting the number of EUS-guided fine-needle passes for diagnosis of pancreatic malignancies. *Gastrointest Endosc* 2000; **51**: 184-190

19 **LeBlanc JK**, Ciaccia D, Al-Assi MT, McGrath K, Imperiale T, Tao LC, Vallery S, DeWitt J, Sherman S, Collins E. Optimal number of EUS-guided fine needle passes needed to obtain a correct diagnosis. *Gastrointest Endosc* 2004; **59**: 475-481

20 **Wallace MB**, Kennedy T, Durkalski V, Eloubeidi MA, Etamad R, Matsuda K, Lewin D, Van Velse A, Hennesey W, Hawes RH, Hoffman BJ. Randomized controlled trial of EUS-guided fine needle aspiration techniques for the detection of malignant lymphadenopathy. *Gastrointest Endosc* 2001; **54**: 441-447

21 **Möller K**, Papanikolaou IS, Toermer T, Delicha EM, Sarbia M, Schenck U, Koch M, Al-Abadi H, Meining A, Schmidt H, Schulz HJ, Wiedenmann B, Rösch T. EUS-guided FNA of solid pancreatic masses: high yield of 2 passes with combined histologic-cytologic analysis. *Gastrointest Endosc* 2009; **70**: 60-69

22 **Iglesias-Garcia J**, Poley JW, Larghi A, Giovannini M, Petrone MC, Abdulkader I, Monges G, Costamagna G, Arcidiacono P, Biermann K, Rindi G, Bories E, Dogloni C, Bruno M, Dominguez-Muñoz JE. Feasibility and yield of a new EUS histology needle: results from a multicenter, pooled, cohort study. *Gastrointest Endosc* 2011; **73**: 1189-1196

23 **Iglesias-Garcia J**, Dominguez-Munoz E, Lozano-Leon A, Abdulkader I, Larino-Noia J, Antunez J, Forteza J. Impact of endoscopic ultrasound-guided fine needle biopsy for diagnosis of pancreatic masses. *World J Gastroenterol* 2007; **13**: 289-293

24 **Voss M**, Hammel P, Molas G, Palazzo L, Dancour A, O' Toole D, Terris B, Degott C, Bernades P, Ruszniewski P. Value of endoscopic ultrasound guided fine needle aspiration biopsy in the diagnosis of solid pancreatic masses. *Gut* 2000; **46**: 244-249

25 **Yasuda I**, Tsurumi H, Omar S, Iwashita T, Kojima Y, Yamada T, Sawada M, Takami T, Moriwaki H, Soehendra N. Endoscopic ultrasound-guided fine-needle aspiration biopsy for lymphadenopathy of unknown origin. *Endoscopy* 2006; **38**: 919-924

26 **Ribeiro A**, Pereira D, Escalón MP, Goodman M, Byrne GE. EUS-guided biopsy for the diagnosis and classification of lymphoma. *Gastrointest Endosc* 2010; **71**: 851-855

27 **Nasuti JF**, Gupta PK, Baloch ZW. Diagnostic value and cost-effectiveness of on-site evaluation of fine-needle aspiration specimens: review of 5,688 cases. *Diagn Cytopathol* 2002; **27**: 1-4

28 **Pellisé Urquiza M**, Fernández-Esparrach G, Solé M, Colomo L, Castells A, Llach J, Mata A, Bordas JM, Piqué JM, Ginès A. Endoscopic ultrasound-guided fine needle aspiration: predictive factors of accurate diagnosis and cost-minimization analysis of on-site pathologist. *Gastroenterol Hepatol* 2007; **30**: 319-324

29 **Cherian PT**, Mohan P, Douiri A, Taniere P, Hejmadi RK, Mahon BS. Role of endoscopic ultrasound-guided fine-needle aspiration in the diagnosis of solid pancreatic and peripancreatic lesions: is onsite cytopathology necessary? *HPB* (*Oxford*) 2010; **12**: 389-395

30 **Klapman JB**, Logrono R, Dye CE, Waxman I. Clinical impact of on-site cytopathology interpretation on endoscopic ultrasound-guided fine needle aspiration. *Am J Gastroenterol* 2003; **98**: 1289-1294

31 **Turner BG**, Cizginer S, Agarwal D, Yang J, Pitman MB, Brugge WR. Diagnosis of pancreatic neoplasia with EUS and FNA: a report of accuracy. *Gastrointest Endosc* 2010; **71**: 91-98

32 **Wiersema MJ**, Vilmann P, Giovannini M, Chang KJ, Wiersema LM. Endosonography-guided fine-needle aspiration biopsy: diagnostic accuracy and complication assessment. *Gastroenterology* 1997; **112**: 1087-1095

33 **Varadarajulu S**, Kilgore ML, Wilcox CM, Eloubeidi MA. Relationship among hospital ERCP volume, length of stay, and technical outcomes. *Gastrointest Endosc* 2006; **64**: 338-347

34 **Cotton PB**. Are low-volume ERCPists a problem in the United States? A plea to examine and improve ERCP practice-NOW. *Gastrointest Endosc* 2011; **74**: 161-166

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BRIEF ARTICLE

Clinical benefit of radiation therapy and metallic stenting for unresectable hilar cholangiocarcinoma

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Abstract

AIM: To determine the efficacy of external beam radiotherapy (EBRT), with or without intraluminal brachytherapy (ILBT), in patients with non-resected locally advanced hilar cholangiocarcinoma.

METHODS: We analyzed 64 patients with locally advanced hilar cholangiocarcinoma, including 25 who underwent resection (17 curative and 8 non-curative), 28 treated with radiotherapy, and 11 who received best supportive care (BSC). The radiotherapy group received EBRT (50 Gy, 30 fractions), with 11 receiving an additional 24 Gy (4 fractions) ILBT by iridium-192 with remote after loading. ILBT was performed using percutaneous transhepatic biliary drainage (PTBD) route. Uncovered metallic stents (UMS) were inserted into non-resected patients with obstructive jaundice, with the exception of four patients who received percutaneous transhepatic biliary drainage only. UMS were placed endoscopically or percutaneously, depending on the initial drainage procedure. The primary endpoints were patient death or stent occlusion. Survival time of patients in the radiotherapy group was compared with that of patients in the resection and BSC groups. Stent patency was compared in the radiotherapy and BSC groups.

RESULTS: No statistically significant differences in patient characteristics were found among the resection, radiotherapy, and BSC groups. Three patients in the radiotherapy group and one in the BSC group did not receive UMS insertion but received PTBD alone; cholangitis occurred after endoscopic stenting, and patients were treated with PTBD. A total of 16 patients were administered additional systemic chemotherapy (5-fluorouracil-based regimen in 9, S-1 in 6, and gemcitabine in 1). Overall survival varied significantly among groups, with median survival times of 48.7 mo in the surgery group, 22.1 mo in the radiotherapy group, and 5.7 mo in the BSC group. Patients who underwent curative resection survived significantly longer than those who were not candidates for surgery (P = 0.0076). Cumulative survival in the radiotherapy group was significantly longer than in the BSC group (P = 0.0031), but did not differ significantly from those in the non-resection group. Furthermore, the median survival time of patients in the radiotherapy group who were considered for possible resection (excluding the seven patients who were not candidates for surgery due to comorbid disease or age) was 25.9 mo. Stent patency was evaluated only in the 24 patients who received a metallic stent. Stent patency was significantly longer in the radiotherapy than in the BSC group (P = 0.0165). Biliary drainage was not eliminated in any patient. To determine the efficacy of ILBT, we compared

survival time and stent patency in the EBRT alone and EBRT plus ILBT groups. However, we found no significant difference in survival time between groups or for stent patencies. Hemorrhagic gastroduodenal ulcers were observed in 5 patients (17.9%), three in the EBRT plus ILBT group and two in the EBRT alone group. Ulcers occurred 5 mo, 7 mo, 8 mo, 16 mo, and 29 mo following radiotherapy. All patients required hospitalization, but blood transfusions were unnecessary. All 5 patients recovered following the administration of anti-ulcer medication.

CONCLUSION: Radiotherapy improved patient prognosis and the patency of uncovered metallic stents in patients with locally advanced hilar cholangiocarcinoma, but ILBT provided no additional benefits.

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Key words: Hilar cholangiocarcinoma; Radiotherapy; Intra-luminal brachytherapy; Biliary metallic stent; Obstructive jaundice

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INTRODUCTION

Hilar cholangiocarcinoma is a relatively rare, slow-growing, late-metastasizing tumor associated with poor patient prognosis[1-7]. The only known effective treatment is surgery, but only a small proportion of patients are suitable candidates. The operation is technically demanding and may be too invasive for high-risk elderly patients.

External beam radiotherapy (EBRT), with or without intraluminal brachytherapy (ILBT), is widely used to treat patients with hilar cholangiocarcinoma. A randomized trial comparing stenting alone with stenting plus radiotherapy showed that both procedures significantly prolonged patient survival and stent patency[8]. However, the relatively long survival time of untreated patients (median, 298 d) suggests that some of these patients may have been resectable. Thus, the effects of radiotherapy alone, and the benefits of ILBT, are not known.

Biliary stenting is a widely-accepted palliative procedure used to treat patients with unresectable hilar cholangiocarcinoma and obstructive jaundice. Patient prognosis, however, is poor, even with the absence of metastasis. A randomized trial showed that uncovered metallic stents remained patent longer than plastic stents in patients with hilar cholangiocarcinoma[9]. The primary cause of uncovered metallic stent occlusion is tumor ingrowth into the mesh[10]. Covered metallic stents prevent tumor ingrowth in patients with distal biliary obstruction and have a longer patency than uncovered metallic stents[11-16]. Although covered metallic stents are not feasible for treating hilar cancer patients because of the complex anatomy of the hilar duct, radiotherapy may be used to prevent tumor ingrowth after placement of an uncovered metallic stent[17].

The aims of this study were therefore twofold: to test the effects of radiotherapy, with or without ILBT, on the survival of hilar cholangiocarcinoma patients who did not undergo surgical resection; and to evaluate whether radiotherapy prolongs stent patency and thus improves patient quality of life.

MATERIALS AND METHODS

Patients

Between 1986 and 2008, 84 patients with hilar cholangiocarcinoma were admitted to the Department of Gastroenterology of Tokyo University Hospital. Of these 84 patients, 20 had metastatic and 64 had locally advanced disease (Figure 1 and Table 1). Of those with locally advanced tumors, 34 fulfilled our resectability criteria, and 25 underwent surgical resection. The remaining 9 patients were not candidates for surgery due to comorbid disease or advanced age. Bile duct cancer was diagnosed by pathological examination, clinical course, or imaging results. Cholangiocarcinoma staging was based on computed tomography, magnetic resonance cholangiopancreatography, or direct cholangiogram using endoscopic retrograde cholangiopancreatography or percutaneous transhepatic biliary drainage (PTBD). In addition, Bismuth's classification was applied[6].

Criteria for resectability

The resectability of each tumor was determined after consultation with the hepato-biliary surgeon. Surgical contraindications included: invasion of the celiac and super mesenteric arteries, biliary invasion of the third branch of the future remnant liver, and invasion of the both hepatic artery and portal vein of the future remnant liver.

Biliary stenting

All patients with locally advanced tumors underwent endoscopic or percutaneous biliary drainage (Table 2). Prophylactic antibiotics were administered intravenously prior to the endoscopic procedure and for 3 d afterwards. An Amsterdam-type plastic stent (7 or 8.5 Fr; Flexima stent, Microvasive, Boston Scientific, Natick, MA, United States) or a nasobiliary drainage tube (Cook Medical Company) was inserted into patients. PTBD using 8 Fr balloon catheters was performed under ultrasonographic and fluoroscopic guidance. Additional stents were inserted or PTBD was performed if jaundice did not improve.

Radiation therapy

Radiotherapy was recommended for patients whose jaun-

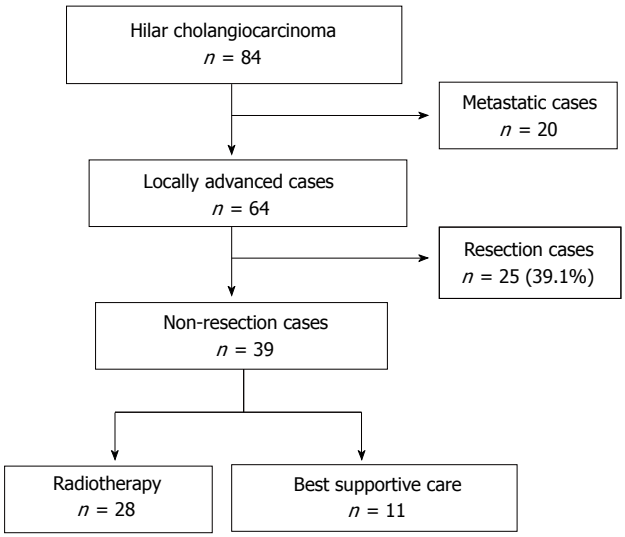

Figure 1 Distribution of patients with hilar cholangiocarcinoma from August 1995 to August 2008.

dice did not improve and who were not candidates for surgery. A total of 28 patients agreed to undergo EBRT (54 Gy, 30 fractions); of these, 11 also underwent percutaneous ILBT (24 Gy, 4 fractions) using a high-dose iridium-192 remote after loading system (Table 2). A metallic stent was inserted into the 11 patients who refused EBRT after their jaundice improved; these patients constituted the best supportive care (BSC) group.

After radiotherapy, a metallic stent was inserted endoscopically or percutaneously, depending on the initial drainage procedure. Some patients received bilateral metallic stents because multiple insertion routes were necessary for internal brachytherapy and unilateral stents produced insufficient drainage.

Follow up

Patient symptoms were assessed and blood tests were performed at monthly intervals in the outpatient clinic. Computed tomography scans were taken every 6 mo (every 2 mo during chemotherapy), with additional scans taken when patients were symptomatic or showed an increase in hepatic-biliary enzymes and bilirubin. Patients received chemotherapy every 2 mo.

Endpoints

The primary endpoints were patient death or stent occlusion through November 2008. Stents were considered patent if the jaundice did not worsen, with or without cholangitis. Patient survival time was measured from the date of diagnosis to the date of death, and stent patency was measured from placement to occlusion or to patient death if the stent remained patent. Survival time of patients in the radiotherapy group was compared with that of patients in the resection and BSC groups. Stent patency was compared in the radiotherapy and BSC groups.

Statistical analysis

Cumulative patient survival and stent patency were analyzed using the Kaplan-Meier method and compared using the log rank test. Patients whose stents were not obstructed were excluded from stent patency analysis. The Mann-Whitney *U*-test was used to compare quantitative variables, and Fisher's exact test was used to analyze qualitative variables. All analyses were performed using StatView 5.0 software (SAS Institute Inc., Cary, NC, United States).

Table 1 Patient characteristics

	Patient group			
	Radiotherapy	BSC	Resection	*P* value
Cases	28	11	25	
Gender (M/F)	14/14	4/7	20/5	
Mean age (yr)	70.1 ± 9.7 (52-86)	74.0 ± 9.0 (61-90)	67.0 ± 9.7 (55-78)	
Reason for non-resection				> 0.9999
Tumor factor	21	9	-	
Patient factor	7	2	-	
Performance status				
0	12	3	17	
1	15	6	8	
2	1	2	0	
TNM stage				0.4953
1a	0	0	7	
1b	2	2	1	
2a	1	0	3	
2b	1	1	8	
3	24	8	6	
Bismuth classification				0.2332
1	3	0	2	
2	6	3	2	
3	7	6	10	
4	12	2	11	

No statistically significant differences in patient characteristics were observed among the resection, radiotherapy, and BSC groups. The mean age of the BSC patients was higher than that of the other groups, but the difference was not significant. TNM: Tumor, node, metastasis; BSC: Best supportive care.

RESULTS

Patient survival

Overall survival varied significantly among groups, with median survival times of 48.7 mo in the surgery group, 22.1 mo in the radiotherapy group, and 5.7 mo in the BSC group (Figure 2). Patients who underwent curative resection survived significantly longer than those who were not candidates for surgery ($P = 0.0076$). Cumulative survival in the radiotherapy group was significantly longer than in the BSC group ($P = 0.0031$), but did not differ significantly from those in the non-resection group (Figure 2). Furthermore, the median survival time of patients in the radiotherapy group who were considered for possible resection (excluding the seven patients who were not candidates for surgery due to comorbid disease or age) was 25.9 mo.

Stent patency

Stent patency was evaluated only in the 24 patients who received a metallic stent (Table 3). Stent patency was significantly longer in the radiotherapy than in the BSC

Table 2 Details of biliary drainage and anti-cancer therapy

	Patient group		
	Radiotherapy	BSC	*P* value
Cases	28	11	
Biliary metallic stenting	24	10	
Drainage area			0.0630
Bilateral	14	2	
Unilateral	10	8	
Insertion route			0.0339
Endoscopic	4	6	
Percutaneous	20	4	
PTBD only	3	1	> 0.9999
No need for drainage	1	0	> 0.9999
Anti-cancer therapy			
Radiotherapy	28	-	
Extra corporeal (50 Gy)	28	-	
Intra bile duct (24 Gy)	11	-	
Additional chemotherapy	16	-	
Gemcitabine	1	-	
S-1	6	-	
5FU-based regimen	9	-	

Three patients in the radiotherapy group and one in the BSC group did not receive a metallic stent but underwent PTBD alone; cholangitis occurred after endoscopic stenting, and patients were treated with PTBD. A total of 16 patients were administered additional systemic chemotherapy, with 9 receiving a 5-fluorouracil (5FU)-based regimen, 6 receiving S-1, and 1 receiving gemcitabine. PTBD: Percutaneous transhepatic biliary drainage; BSC: Best supportive care.

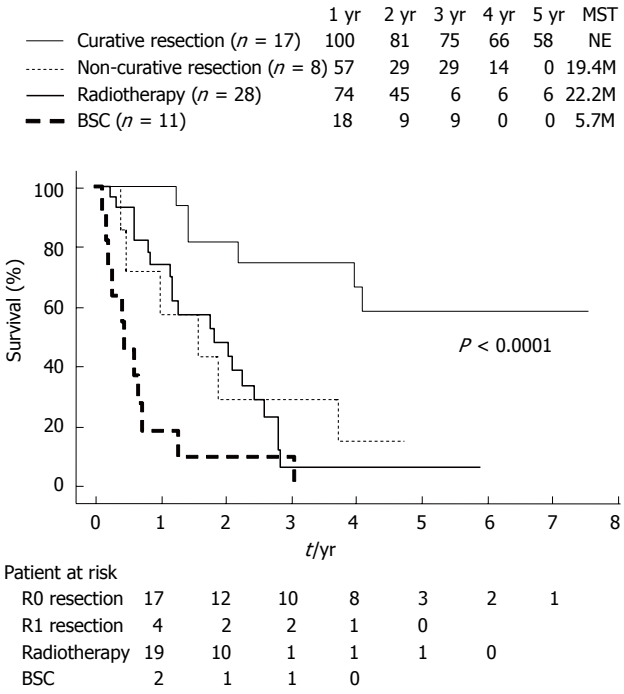

Figure 2 Cumulative survival rate and median survival time of patients who received curative or non-curative resection, radiotherapy or best supportive care. Cumulative survival times were calculated using the Kaplan-Meier method and compared using the log rank test. BSC: Best supportive care; MST: Median survival time; NE: Not evaluable.

group (P = 0.0165; Figure 3). Biliary drainage was not eliminated in any patient.

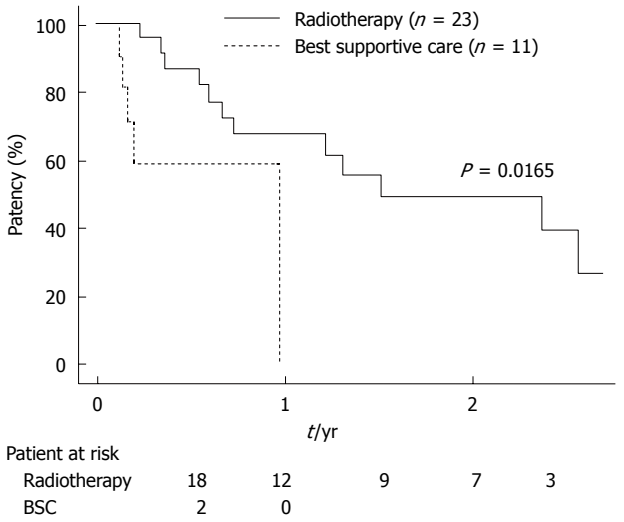

Figure 3 Cumulative metallic stent patency. Stents were patent significantly longer in the radiotherapy than in the BSC group (P = 0.0165). BSC: Best supportive care.

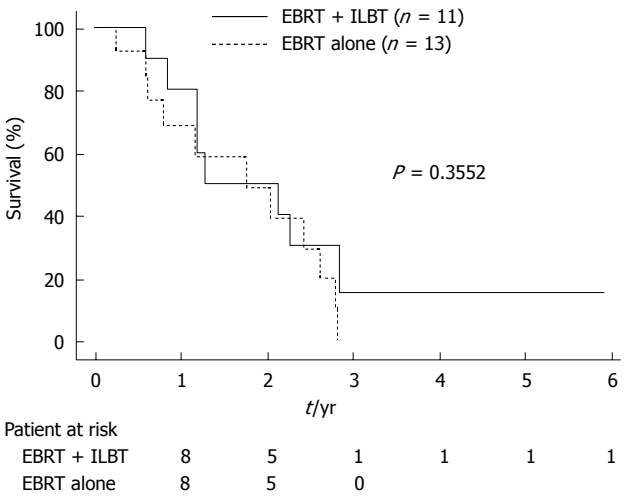

Figure 4 Cumulative survival times of patients who received external beam radiotherapy alone and those who received external beam radiotherapy plus intra bile ductal radiotherapy (intraluminal brachytherapy). There was no significant difference in survival between groups. EBRT: External beam radiotherapy; ILBT: Intraluminal brachytherapy.

Additive effect of ILBT to EBRT

To determine the efficacy of ILBT, we compared survival time and stent patency in the EBRT alone and EBRT plus ILBT groups. However, we found no significant difference in survival time between groups (Figure 4) or for stent patencies.

Complications of radiotherapy

Hemorrhagic gastroduodenal ulcers were observed in 5 patients (17.9%), three in the EBRT plus ILBT group and two in the EBRT alone group. Ulcers occurred 5 mo, 7 mo, 8 mo, 16 mo, and 29 mo following radiotherapy. All patients required hospitalization, but blood transfusions were unnecessary. All 5 patients recovered follow-

Table 3 Results of metallic stenting

	Patient group		
	Radiotherapy	BSC	*P* value
Cases	23	11	
Mean stent patency (d)[1]	604 ± 78.3	235 ± 53.3	0.0038
Median stent patency (d)[1]	557	358	
Stent obstruction (%)	12 (56.5)	4 (36.4)	0.4646
Mean patent period	329.2 ± 234.8	136.8 ± 147.1	0.1472
Cause			
Tumor ingrowth	9	1	0.1133
Tumor overgrowth	1	1	0.5490
Sludge	2	2	0.5799

[1]Mean and median stent patency were calculated using the Kaplan-Meier method and compared using the log rank test. Stent patency was significantly longer in the radiotherapy than in the BSC group. Mean patent period was longer in the radiotherapy than in the BSC group, but the difference was not significant. BSC: Best supportive care.

ing the administration of anti-ulcer medication.

DISCUSSION

Surgical resection is the only known cure for hilar cholangiocarcinoma. The surgery is highly invasive and recommended for only a small percentage of patients; thus, alternative treatments are necessary. Our results suggest that radiotherapy prolongs survival in patients who cannot undergo resection. Survival was significantly longer in patients who received radiotherapy than in those who received BSC, but was similar to that of patients who underwent non-curative resection.

Radiotherapy has been shown to improve survival times of patients with locally advanced hilar cholangiocarcinoma who cannot undergo curative resection. For example, a randomized trial reported that median survival times were 12.9 mo in patients who received both EBRT and ILBT and 9.3 mo in patients did not receive radiotherapy[8]. A second study found that median survival in patients who received chemoradiotherapy (EBRT plus 5-fluorouracil) was 22 mo, but that study did not include an untreated control group[18]. A retrospective analysis found that the median survival time in patients with extrahepatic cholangiocarcinoma who received EBRT plus ILBT was 9 mo, compared with 5 mo in patients treated with EBRT alone[19]. This wide range in survival times may reflect differences in patient backgrounds. Most reports did not describe the percentage of patients with resectable tumors, the reasons that they were not resected, or the resection criteria; thus, it is difficult to generalize from these results. In contrast, we have stated the inclusion criteria for non-surgical treatment. Moreover, in our radiotherapy group, the median survival of non-resected patients considered for possible resection was 25.9 mo, longer than previously reported survival times.

We observed no significant differences between the radiotherapy and BSC groups in patient background, including the reasons for not undergoing resection and performance score. We expected that selection bias may have resulted in longer median survival of the radiotherapy than of the BSC group, but we found no significant difference in background between these two groups. Our results therefore provide additional evidence suggesting that radiotherapy increases survival time in patients with locally advanced hilar cholangiocarcinoma.

Although surgery is the only known cure for cholangiocarcinoma, we found that median survival of the radiotherapy group did not differ significantly from that of the non-curative resection group. This finding supports the survival benefits of radiotherapy for patients with locally advanced hilar cholangiocarcinoma. Thus, radiotherapy is a treatment option for patients in poor condition or those with highly invasive tumors.

Biliary stenting is a widely accepted palliative treatment for non-resected patients with biliary obstruction, with uncovered metallic stents maintaining patency longer than plastic stents[9]. The primary cause of obstruction in patients who receive an uncovered metallic stent is tumor ingrowth into the stent mesh. In a previous randomized trial, we prevented distal biliary obstruction by inhibiting tumor ingrowth and found that a covered metallic stent was patent longer than an uncovered stent[11]. However, covered metallic stents cannot be used in patients with hilar obstruction, and it is necessary to develop strategies to prevent tumor ingrowth of hilar lesions. Stents remained patent significantly longer in the radiotherapy group than in the BSC group (stenting alone); suggesting that local tumor control using radiotherapy prolongs stent patency. Previous studies of the efficacy of metallic stents in the treatment of hilar cholangiocarcinoma did not use radiotherapy, and studies investigating the survival benefits of radiotherapy have not reported results of metallic stenting. Thus, no evidence was available to determine the efficacy of radiotherapy in improving stent patency.

Multiple laser-cut-type Nitinol stents were found to have a mean patency of 150 d[20], with Wallstents having a median patency of 169 d[21]. A comparison of the patency of metallic stents inserted endoscopically and percutaneously showed that the median stent patency of these two groups was 9.8 mo and 11.0 mo, respectively[22]. In comparison, we found that median and mean stent patency in our radiotherapy group were 557 d and 604 d, respectively. The length of time the stents remained patent suggests that radiotherapy had a significant effect on patency.

We found that the addition of ILBT to EBRT did not improve patient survival or stent patency. Stent patency is a surrogate indicator of local anti-tumor effectiveness, because the main cause of stent occlusion is tumor ingrowth through the stent mesh. ILBT requires an additional treatment period and multiple PTBD insertions. Since it did not improve patient survival or stent patency, we do not recommend this additional treatment. A retrospective study of 31 patients found that, although additional ILBT improved the 2-year survival rate, it had no effect on cumulative survival time[20]. No randomized studies to date

have compared the effect on survival of radiotherapy with and without ILBT. Thus, the efficacy of additional ILBT remains unclear.

Photodynamic therapy is a promising local anti-tumor therapy for patients with non-resectable hilar cholangiocarcinoma, with median survival times of patients who did and not receive photodynamic therapy of 493 d and 98 d, respectively, a difference that was statistically significant[23]. Although photodynamic therapy can be used to treat local tumors extending along the bile duct, it cannot be used to treat large tumors that have invaded other organs, and it cannot be used to treat loco-regional lymph node metastasis because it is performed in the bile duct. The combination of photodynamic therapy with EBRT may be a more feasible treatment option.

The major limitation of this study was its retrospective design. Moreover, despite all included patients having unresectable, locally advanced tumors, the characteristics of the radiation and BSC groups differed. Since there were no selection criteria for the BSC group, the differences in outcomes between this group and the radiation group may be due not only to the effects of radiation, but to patient condition as well.

In conclusion, radiotherapy may improve the prognosis of patients with non-resected, locally advanced hilar cholangiocarcinoma and may increase the patency of uncovered metallic stents. ILBT provides no benefit for these patients.

COMMENTS

Background

Hilar cholangiocarcinoma is a relatively rare, slow-growing and late-metastasizing tumor associated with poor patient prognosis. Surgery is the only known curative treatment, but it may be technically difficult and too invasive for high-risk elderly patients. The treatment of patients with unresectable tumors has not been established.

Research frontiers

Biliary stenting and radiotherapy, including external beam radiotherapy (EBRT) with or without intraluminal brachytherapy (ILBT), are widely used to treat patients with locally advanced hilar cholangiocarcinoma. These treatments, however, are not well established, although a recent randomized study showed good results for photodynamic therapy.

Innovations and breakthroughs

Several recent studies have shown the efficacy of radiation therapy in patients with locally advanced hilar cholangiocarcinoma. In many of those patients, however, the tumors may have been resectable. The authors clarified the reasons for non-resection of the patients and compared outcomes with resected patients. The authors found that radiation therapy effectively prolonged patient survival and stent patency. They also showed that additional ILBT did not affect survival or stent patency.

Applications

EBRT is indicated for patients with locally advanced hilar cholangiocarcinoma, with similar survival times as patients who underwent non-curative resection. Patients who undergo uncovered metallic stent placement should be considered for EBRT to prolong stent patency and survival.

Terminology

EBRT is a conventional radiation method, and ILBT is performed with iridium wire through a PTBD catheter using an after loading system. A metallic stent is a braided metallic wire or tubular nitinol cut with a laser. Metallic stents have self-expandability and are mounted onto a thin delivery system (6-9Fr). This type of stent, however, tends to be occluded by tumor ingrowth into the stent mesh.

Peer review

As stated by the authors, the limitation of the study is that it is a retrospective study and that the groups are not the results of a randomization but the consequence of different situations such not being suitable for surgery. In any case, the paper is a well presented study on a very difficult subject and is worthy of publication.

REFERENCES

1 **Klatskin G**. Adenocarcinoma of the hepatic duct at its bifurcation within the porta hepatis. An unusual tumor with distinctive clinical and pathological features. *Am J Med* 1965; **38**: 241-256

2 **Bismuth H**, Nakache R, Diamond T. Management strategies in resection for hilar cholangiocarcinoma. *Ann Surg* 1992; **215**: 31-38

3 **Inouye AA**, Whelan TJ. Carcinoma of the extrahepatic bile ducts: a ten year experience in Hawaii. *Am J Surg* 1978; **136**: 90-95

4 **Blumgart LH**, Hadjis NS, Benjamin IS, Beazley R. Surgical approaches to cholangiocarcinoma at confluence of hepatic ducts. *Lancet* 1984; **1**: 66-70

5 **Langer JC**, Langer B, Taylor BR, Zeldin R, Cummings B. Carcinoma of the extrahepatic bile ducts: results of an aggressive surgical approach. *Surgery* 1985; **98**: 752-759

6 **Bismuth H**, Castaing D, Traynor O. Resection or palliation: priority of surgery in the treatment of hilar cancer. *World J Surg* 1988; **12**: 39-47

7 **Seyama Y**, Kubota K, Sano K, Noie T, Takayama T, Kosuge T, Makuuchi M. Long-term outcome of extended hemihepatectomy for hilar bile duct cancer with no mortality and high survival rate. *Ann Surg* 2003; **238**: 73-83

8 **Válek V**, Kysela P, Kala Z, Kiss I, Tomásek J, Petera J. Brachytherapy and percutaneous stenting in the treatment of cholangiocarcinoma: a prospective randomised study. *Eur J Radiol* 2007; **62**: 175-179

9 **Wagner HJ**, Knyrim K, Vakil N, Klose KJ. Plastic endoprostheses versus metal stents in the palliative treatment of malignant hilar biliary obstruction. A prospective and randomized trial. *Endoscopy* 1993; **25**: 213-218

10 **De Palma GD**, Pezzullo A, Rega M, Persico M, Patrone F, Mastantuono L, Persico G. Unilateral placement of metallic stents for malignant hilar obstruction: a prospective study. *Gastrointest Endosc* 2003; **58**: 50-53

11 **Isayama H**, Komatsu Y, Tsujino T, Sasahira N, Hirano K, Toda N, Nakai Y, Yamamoto N, Tada M, Yoshida H, Shiratori Y, Kawabe T, Omata M. A prospective randomised study of "covered" versus "uncovered" diamond stents for the management of distal malignant biliary obstruction. *Gut* 2004; **53**: 729-734

12 **Isayama H**, Komatsu Y, Tsujino T, Yoshida H, Tada M, Shiratori Y, Kawabe T, Omata M. Polyurethane-covered metal stent for management of distal malignant biliary obstruction. *Gastrointest Endosc* 2002; **55**: 366-370

13 **Nakai Y**, Isayama H, Komatsu Y, Tsujino T, Toda N, Sasahira N, Yamamoto N, Hirano K, Tada M, Yoshida H, Kawabe T, Omata M. Efficacy and safety of the covered Wallstent in patients with distal malignant biliary obstruction. *Gastrointest Endosc* 2005; **62**: 742-748

14 **Isayama H**, Kawabe T, Nakai Y, Ito Y, Togawa O, Kogure H, Yashima Y, Yagioka H, Matsubara S, Sasaki T, Sasahira N, Hirano K, Tsujino T, Tada M, Omata M. Management of distal malignant biliary obstruction with the ComVi stent, a new covered metallic stent. *Surg Endosc* 2010; **24**: 131-137

15 **Kubota Y,** Mukai H, Nakaizumi A, Tanaka K, Okabe Y, Sakagami T, Kitano M, Mitsufuji S, Shirasaka D, Kikuchi E, Koyama S, Yazumi S, Shiba M, Yasuda K. Covered Wallstent for palliation of malignant common bile duct stricture: prospective multicenter evaluation. *Digest Endosc* 2005; **17**:

218-223
16 **Kahaleh M**, Tokar J, Conaway MR, Brock A, Le T, Adams RB, Yeaton P. Efficacy and complications of covered Wallstents in malignant distal biliary obstruction. *Gastrointest Endosc* 2005; **61**: 528-533
17 **Bowling TE**, Galbraith SM, Hatfield AR, Solano J, Spittle MF. A retrospective comparison of endoscopic stenting alone with stenting and radiotherapy in non-resectable cholangiocarcinoma. *Gut* 1996; **39**: 852-855
18 **Deodato F**, Clemente G, Mattiucci GC, Macchia G, Costamagna G, Giuliante F, Smaniotto D, Luzi S, Valentini V, Mutignani M, Nuzzo G, Cellini N, Morganti AG. Chemoradiation and brachytherapy in biliary tract carcinoma: long-term results. *Int J Radiat Oncol Biol Phys* 2006; **64**: 483-488
19 **Shin HS**, Seong J, Kim WC, Lee HS, Moon SR, Lee IJ, Lee KK, Park KR, Suh CO, Kim GE. Combination of external beam irradiation and high-dose-rate intraluminal brachytherapy for inoperable carcinoma of the extrahepatic bile ducts. *Int J Radiat Oncol Biol Phys* 2003; **57**: 105-112
20 **Kawamoto H**, Tsutsumi K, Harada R, Fujii M, Kato H, Hirao K, Kurihara N, Nakanishi T, Mizuno O, Ishida E, Ogawa T, Fukatsu H, Sakaguchi K. Endoscopic deployment of multiple JOSTENT SelfX is effective and safe in treatment of malignant hilar biliary strictures. *Clin Gastroenterol Hepatol* 2008; **6**: 401-408
21 **Cheng JL**, Bruno MJ, Bergman JJ, Rauws EA, Tytgat GN, Huibregtse K. Endoscopic palliation of patients with biliary obstruction caused by nonresectable hilar cholangiocarcinoma: efficacy of self-expandable metallic Wallstents. *Gastrointest Endosc* 2002; **56**: 33-39
22 **Paik WH**, Park YS, Hwang JH, Lee SH, Yoon CJ, Kang SG, Lee JK, Ryu JK, Kim YT, Yoon YB. Palliative treatment with self-expandable metallic stents in patients with advanced type III or IV hilar cholangiocarcinoma: a percutaneous versus endoscopic approach. *Gastrointest Endosc* 2009; **69**: 55-62
23 **Ortner ME**, Caca K, Berr F, Liebetruth J, Mansmann U, Huster D, Voderholzer W, Schachschal G, Mössner J, Lochs H. Successful photodynamic therapy for nonresectable cholangiocarcinoma: a randomized prospective study. *Gastroenterology* 2003; **125**: 1355-1363

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BRIEF ARTICLE

Comparison of percutaneous transhepatic portal vein embolization and unilateral portal vein ligation

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Abstract

AIM: To compare the effect of percutaneous transhepatic portal vein embolization (PTPE) and unilateral portal vein ligation (PVL) on hepatic hemodynamics and right hepatic lobe (RHL) atrophy.

METHODS: Between March 2005 and March 2009, 13 cases were selected for PTPE (n = 9) and PVL (n = 4) in the RHL. The PTPE group included hilar bile duct carcinoma (n = 2), intrahepatic cholangiocarcinoma (n = 2), hepatocellular carcinoma (n = 2) and liver metastasis (n = 3). The PVL group included hepatocellular carcinoma (n = 2) and liver metastasis (n = 2). In addition, observation of postoperative hepatic hemodynamics obtained from computed tomography and Doppler ultrasonography was compared between the two groups.

RESULTS: Mean ages in the two groups were 58.9 ± 2.9 years (PVL group) *vs* 69.7 ± 3.2 years (PTPE group), which was a significant difference (P = 0.0002). Among the indicators of liver function, including serum albumin, serum bilirubin, aspartate aminotransferase, alanine aminotransferase, platelets and indocyanine green retention rate at 15 min, no significant differences were observed between the two groups. Preoperative RHL volumes in the PTPE and PVL groups were estimated to be 804.9 ± 181.1 mL and 813.3 ± 129.7 mL, respectively, with volume rates of 68.9% ± 2.8% and 69.2% ± 4.2%, respectively. There were no significant differences in RHL volumes (P = 0.83) and RHL volume rates (P = 0.94), respectively. At 1 mo after PTPE or PVL, postoperative RHL volumes in the PTPE and PVL groups were estimated to be 638.4 ± 153.6 mL and 749.8 ± 121.9 mL, respectively, with no significant difference (P = 0.14). Postoperative RHL volume rates in the PTPE and PVL groups were estimated to be 54.6% ± 4.2% and 63.7% ± 3.9%, respectively, which was a significant difference (P = 0.0056). At 1 mo after the operation, the liver volume atrophy rate was 14.3% ± 2.3% in the PTPE group and 5.4% ± 1.6% in the PVL group, which was a significant difference (P = 0.0061).

CONCLUSION: PTPE is a more effective procedure than PVL because PTPE is able to occlude completely the portal branch throughout the right peripheral vein.

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Key words: Percutaneous transhepatic portal vein embolization; Portal vein ligation; Liver atrophy; Future liver remnant; Two-stage hepatectomy

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INTRODUCTION

Postoperative liver failure may be induced by major hepatectomy, whereby more than 60%-70% of total liver volume is planned for resection in cases such as hilar bile duct carcinoma, hepatocellular carcinoma and liver metastasis. To reduce the risk of postoperative liver failure due to an insufficient volume of functional liver, the well-established percutaneous transhepatic portal vein embolization (PTPE) procedure can be performed prior to major hepatectomy[1-6]. Unilateral portal vein ligation (PVL) is an alternative method that requires laparotomy[7,8]. Although PTPE has since its introduction become the more popular procedure of the two, PVL remains a viable option during the initial stage of a two-stage hepatectomy procedure (TSHP) for cases such as bilobar multiple liver metastases[9-11]. Both of these techniques occlude the unilateral portal vein, with the aim of inducing atrophy of the ipsilateral liver and thus inducing hypertrophy of the future liver remnant (FLR). In the present study, we retrospectively compared the effectiveness of the two techniques by evaluating the postoperative atrophy rate and hemodynamics of the right hepatic lobe (RHL).

MATERIALS AND METHODS

Patient characteristics of the PTPE and PVL groups

Between March 2005 and March 2009, nine patients were selected for PTPE and four for PVL. The mean patient age was 69.7 ± 3.2 years for the PTPE group and 58.9 ± 2.9 years for the PVL group (P = 0.0002). The clinical characteristics of the patients included hilar bile duct carcinoma (n = 2), intrahepatic cholangiocarcinoma (n = 2), hepatocellular carcinoma (n = 2) and liver metastasis (n = 3) in the PTPE group, and the PVL group had hepatocellular carcinoma (n = 2) and liver metastasis (n = 2). The PTPE group included one patient with hepatitis C, one with hepatitis B, and seven positive for hepatitis B virus (HBV) antigen but negative for hepatitis C virus (HCV) antibody. The PVL group included one patient with hepatitis C, one with hepatitis B, and two positive for HBV antigen and negative for HCV antibody.

PTPE cases

Two patients were diagnosed with hilar bile duct carcinoma, based on findings of obstructive jaundice. Endoscopic nasobiliary drainage was first performed. Before conducting an extended right lobectomy, PTPE was performed because the liver volumes to be resected were 70.8% and 70.2% in the two patients.

In the two cases of intrahepatic cholangiocarcinoma, the tumors were 10 cm and 8 cm in diameter, and both were adjacent to the hilar plate. These two patients also underwent a preoperative PTPE because of planned liver resection volumes of 74.2% and 70.5% respectively.

In the two patients with hepatocellular carcinoma, the tumors were 4.0 cm and 3.5 cm in diameter and adjacent to the right Glisson's capsule. In addition, both the tumors recurred after the transarterial embolization procedure. The liver volume to be resected in these cases was 68.3% and 66.1%, respectively. In addition, indocyanine green retention rate at 15 min (ICG R15) was 17% and 18%, respectively, indicating functional liver impairment. Because of these factors, PTPE was performed prior to resection.

In the three patients with liver metastases, multiple lesions in the right lobes were noted after the initial round of chemotherapy. Preoperative PTPE was scheduled because the liver volumes to be resected in the three patients were 65.2%, 66.6% and 68.8%.

PVL cases

Patients with hepatocellular carcinoma in the PVL group presented with lymph node metastasis. The tumors adjacent to the right Glisson's capsule were 5 cm and 4 cm in diameter with ICG R15 of 38.8% and 15.2%, respectively. The planned liver resection volumes were 65.1% and 69.8%, respectively. Thus, PVL was performed on the right portal branch only during the implementation of lymphadenectomy.

Regarding the two liver metastasis cases, the patients had undergone chemotherapy and developed multiple synchronous liver metastases to both lobes, disseminated from ascending colon cancer. Because the liver resection volumes were 66.9% and 74.8%, the right portal vein was ligated. In addition, right colectomy and partial resection of the left hepatic lobe were also performed in both patients.

Indications for resection

Resectability criteria included an FLR of ⩽ 30% of the total liver volume, whereas this criterion was ⩽ 35% for the patients who had liver cirrhosis or underwent neoadjuvant chemotherapy. In addition, the following equation established by Yamanaka *et al*[12,13] and Okamoto *et al*[14] was used to predict posthepatectomy liver failure: Y = -110 + 0.942 × resection rate (%) + 1.36 × ICG retention rate (%) + 1.17 × patient's age + 5.94 × ICG maximal removal rate (mg/kg per minute). With this equation, the patients who had a calculated Y value > 50 points were deemed unresectable.

PVL and PTPE techniques

For all patients, PTPE or PVL was performed on the RHL. PVL was indicated for patients who were to undergo laparotomy for lymphadenectomy or right colectomy. In PTPE, the umbilical portion of the portal vein was punctured, and the right branch of the portal vein was embolized using a mixture of fibrin glue (Beriplast P; CSL Behring, Tokyo, Japan) and iodized oil (Lipiodol; Guerbet, Aulnay-sous-Bois, France). In the PVL cases, preoperative multidetector row computed tomography (CT) was routinely performed to check for the presence of anatomical variants of the right portal vein. The right branch of the portal vein was intraoperatively isolated and ligated. After each method, Doppler ultrasonography was used to confirm that portal blood flow had

Table 1 Clinical characteristics of the study population (mean ± SD)

Variables	PTPE group (n = 9)	PVL group (n = 4)	P value
Age (yr)	69.7 ± 3.2	58.9 ± 2.9	0.0002
Sex (male:female)	7:2	3:1	
Background (HCV:HBV:NBNC)	1:1:7	1:1:2	
Albumin (g/dL)	3.9 ± 0.4	4.0 ± 0.4	0.61
Bilirubin (mg/dL)	0.9 ± 0.4	1.2 ± 0.7	0.35
AST (IU/L)	30.6 ± 12.2	32.3 ± 8.5	0.47
ALT (IU/L)	35.9 ± 29.1	29.3 ± 15.6	0.92
WBC (/mm^3)	6744 ± 3109	5228 ± 1973	0.41
PLT (× 10^4/mL)	22.1 ± 11.0	19.3 ± 9.9	0.76
PT (%)	84.1 ± 10.5	84.5 ± 12.2	0.86
ICG 15 (%)	10.7 ± 7.4	17.5 ± 13.2	0.43
Child pugh (A:B:C)	7:2:0	3:1:0	

PTPE: Percutaneous transhepatic portal vein embolization; PVL: Portal vein ligation; HCV: Hepatitis C virus; HBV: Hepatitis B virus; NBNC: Hepatitis B surface antigen and HCV antibody negative; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; WBC: White blood cells; PLT: Platelet count; PT: Prothrombin time; ICG 15: Indocyanine green retention rate at 15 min.

been occluded in the ligated lobe and that it had been sustained in the FLR.

Follow-up

No patients had any postoperative complications. Although a slight postoperative decline in liver function was noted in some patients, all improved with conservative treatments.

Statistical analysis

Each patient underwent CT volumetry 1 mo before and after each procedure to evaluate volume changes in the RHL, and the values were compared between the two groups. The RHL atrophy rate was estimated by subtracting the RHL volume rate at 1 mo after PTPE or PVL from the preoperative RHL volume rate. The values in each group were compared using the Mann-Whitney test. All analyses were performed using statistical software (JMP 8.0.2 Macintosh; SAS Institute, Japan). Differences were considered statistically significant at $P < 0.05$.

RESULTS

Clinical characteristics of the study population

The mean age of patients was significantly higher in the PTPE group (58.9 ± 2.9 years *vs* 69.7 ± 3.2 years in the PTPE group), which was a significant difference (P = 0.0002). Among the indicators of liver function, including serum albumin, serum bilirubin, asparate aminotransferase, alanine aminotransferase, platelets and ICG R15, no significant differences were observed between the two groups (Table 1).

Volume change of RHL

Preoperative RHL volumes in the PTPE and PVL groups were estimated to be 804.9 ± 181.1 mL and 813.3 ± 129.7 mL, respectively, with volume rates of 68.9% ± 2.8% and 69.2% ± 4.2%, respectively. There were no significant statistical differences in RHL volumes (P = 0.83) and RHL volume rates (P = 0.94).

Table 2 Right hepatic lobe atrophy rate 1 mo after percutaneous transhepatic portal vein embolization or portal vein ligation (mean ± SD)

Variables	PTPE group (n = 9)	PVL group (n = 4)	P value
Preoperative RHL volume (mL)	804.9 ± 181.1	813.3 ± 129.7	0.83
Preoperative RHL volume rate (%)	68.9 ± 2.8	69.2 ± 4.2	0.94
Postoperative RHL volume (mL)	638.4 ± 153.6	749.8 ± 121.9	0.14
Postoperative RHL volume rate (%)	54.6 ± 4.2	63.7 ± 3.9	0.0056
RHL atrophy rate 1 mo postoperation (%)	14.3 ± 2.3	5.4 ± 1.6	0.0061

PTPE: Percutaneous transhepatic portal vein embolization; PVL: Portal vein ligation; RHL: Right hepatic lobe.

At 1 mo after PTPE or PVL, postoperative RHL volumes in the PTPE and PVL groups were estimated to be 638.4 ± 153.6 mL and 749.8 ± 121.9 mL, respectively, which was not a significant difference (P = 0.14). Postoperative RHL volume rates in the PTPE and PVL groups were estimated to be 54.6% ± 4.2% and 63.7% ± 3.9%, respectively, which was a significant difference (P = 0.0056). At 1 mo postoperatively, the liver volume atrophy rate was 14.3% ± 2.3% in the PTPE group and 5.4% ± 1.6% in the PVL group, which was a significant difference (P = 0.0061) (Table 2).

With respect to the findings from imaging, postoperative CT and Doppler ultrasonography data confirmed residual peripheral portal inflow in the right branch of the ligated portal vein in two cases in the PVL group. In contrast, portal venous flow was confirmed as completely occluded in the PTPE group.

Liver resection and postoperative course

PTPE successfully facilitated liver resection for all nine patients, whereas among the four patients who underwent PVL, two with hepatocellular carcinoma remained unresectable after the procedure. An extended right lobectomy was performed for two hilar bile duct carcinomas and two intrahepatic cholangiocellular carcinomas. A right lobectomy was performed in all the other resectable cases. The 11 patients showed no postoperative complications.

DISCUSSION

Through experimentation on rabbits in 1920, Rous *et al*[15] proved that PVL could induce atrophy of the ipsilateral hepatic lobe and hypertrophy of the FLR lobe. Since then, this technique has been clinically applied by Honjo *et al*[16] and has recently been adopted for TSHP for bilobar multiple liver metastases. In the first stage, partial resec-

Figure 1 Computed tomography showing a case of hilar bile duct carcinoma where the right hepatic lobe volume rate decreased from 68.3% to 50.0% at 1 mo after percutaneous transhepatic portal vein embolization. A: Before percutaneous transhepatic portal vein embolization (PTPE) [right hepatic lobe (RHL) volume rate: 68.3%]; B: After PTPE (RHL volume rate: 50.0%); C: The portal vein in the RHL was completely occluded after PTPE.

Figure 2 Computed tomography showing a case of liver metastasis where the right hepatic lobe volume rate decreased from 66.9% to 60.4%. A: Before portal vein ligation (PVL) [right hepatic lobe (RHL) volume rate: 66.9%]; B: After PVL (RHL volume rate: 60.4%); C: The right portal branch was intraoperatively ligated (arrow).

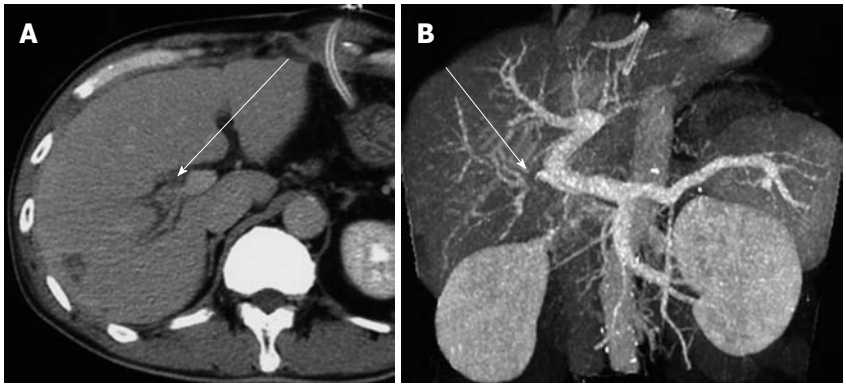

Figure 3 Postoperative computed tomography confirming a degree of residual portal venous flow from the periphery to the ligation point (arrow). A: Axial image; B: Multiplanar reconstruction image.

tion or ablation is performed on the FRL, and PVL is subsequently performed to induce atrophy in the hemiliver to be resected, in preparation for the planned major hepatectomy[17-20]. PTPE has been advocated as a technique for inducing atrophy of the ipsilateral liver without the need for a laparotomy, and is commonly performed for cases such as hilar bile duct carcinoma and hepatocellular carcinoma, in which a substantial liver volume is planned for resection[21-23]. Although PVL and PTPE are both techniques that occlude the portal vein, they differ in approach. PTPE is performed by percutaneously injecting embolic materials, whereas PVL is performed by a laparotomy to ligate the first-order branch of the portal vein. To date, several studies have demonstrated the safety and effectiveness of the two techniques individually; however, there are not enough data available from comparative studies reporting the relative efficacies of the two techniques. In the present study, we compared the atrophic effect and postoperative hepatic hemodynamics associated with PTPE and PVL.

In our study, the PTPE group demonstrated a significantly higher rate of postoperative liver volume atrophy than the PVL group 1 mo after hepatectomy. The mean liver volume atrophy rate was 14.3% ± 2.3% in the PTPE group and 5.4% ± 1.6% in the PVL group (Figures 1 and 2). The CT and Doppler ultrasound findings may explain the reason for this difference. It was confirmed that portal venous flow was completely occluded due to the use of embolic materials acting throughout the peripheral portal vein in the PTPE group. However, portal vein venous flow continued at peripheral sites away from the ligation point in two cases in the PVL group (Figure 3). Residual flow was observed from the anterior to the posterior branch (Figure 4).

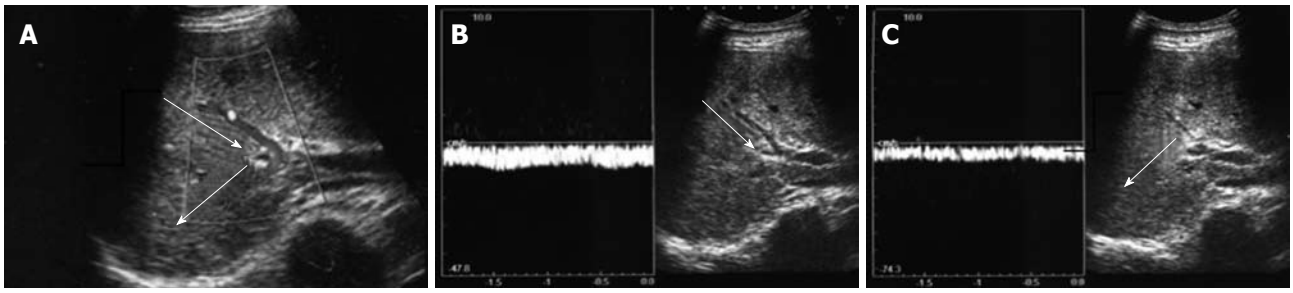

Figure 4 Doppler ultrasound findings on hemodynamics. A: Color Doppler ultrasound confirming portal venous flow from the anterior to the posterior branch (arrow); B: Pulse Doppler ultrasound confirming hepatofugal venous flow in the anterior branch (arrow); C: Pulse Doppler ultrasound confirming hepatopetal venous flow in the posterior branch (arrow).

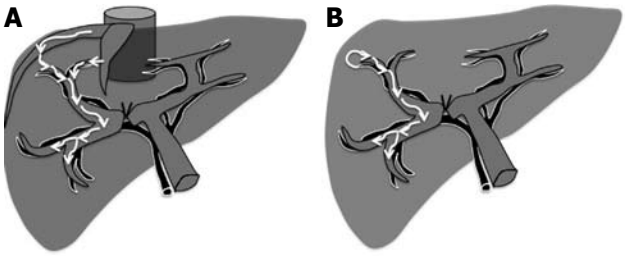

Figure 5 Hemodynamics during portal vein ligation procedure. A: The residual portal venous flow may be explained as backflow from the hepatic vein; B: The presence of an arterioportal shunt may be another reason for the residual portal venous flow.

Two explanations can be proposed for the peripheral portal blood flow observed despite the portal vein being ligated at its central site. First, the observed peripheral portal blood flow could be backflow from the ipsilateral hepatic vein (Figure 5A). Normally, portal vein pressure and hepatic venous pressure are approximately equal at 100-150 mmH_2O. However, ligating the first-order branch of the portal vein may decrease peripheral portal vein pressure, causing a relative pressure elevation in the hepatic vein and resulting in backflow from the hepatic vein. This backflow was significant for cases where tumors had compressed or obstructed hepatic veins in the presence of high hepatic venous pressure. A second possible explanation for the observed peripheral portal blood flow is related to the arterioportal shunt (AP shunt) (Figure 5B). Blood inflow *via* the AP shunt diminishes the effectiveness of the technique by allowing some blood to flow in the first-order branch of the portal vein. Consequently, when the AP shunt is preoperatively observed, PVL should not be the preferred treatment option.

In contrast to the PVL group, postoperative CT data confirmed the presence of embolic materials in the peripheral portal vein in all PTPE patients. In addition, postoperative Doppler ultrasound showed no residual blood in the portal vein, leading us to conclude the superiority of PTPE over PVL. However, PVL is currently more often performed in TSHP cases requiring portal vein occlusion. Time between the first stage and PTPE should be minimized because of possible cancer progression[24,25]. Therefore, we recommend PTPE for the first part of TSHP.

In conclusion, the small sample size and the retrospective nature of this study are limitations towards obtaining more conclusive results, compared to a prospective study with a larger population. However, our results show that PTPE can more effectively and rapidly achieve atrophy of ipsilateral liver volume and consequently induce compensatory FLR volume hypertrophy, compared with PVL.

COMMENTS

Background

Postoperative liver failure may be induced because of the insufficient remnant liver volume after major hepatectomy where more than 60%-70% of the total liver volume is resected in such cases as hilar bile duct carcinoma or liver metastasis. To achieve safer major hepatectomy, ligation or embolization of the portal vein is preliminarily performed to induce atrophy of the ipsilateral liver and hypertrophy of the future remnant liver.

Research frontiers

To occlude unilateral portal vein, either portal vein ligation (PVL) ligating the unilateral portal vein or percutaneous transhepatic portal vein embolization (PTPE) injecting embolic agent to the unilateral portal vein through catheter is performed. PTPE is a procedure advocated after PVL, and PVL cases have decreased in number since the introduction of PTPE. However, as two-stage hepatectomy procedure (TSHP) for liver metastasis has recently become a more popular procedure, PVL has been again increasingly performed in combination with the first resection of TSHP.

Innovations and breakthroughs

The liver atrophy rate of unilateral lobe was compared between the PTPE (n = 9) and PVL groups (n = 4) 1 mo after each procedure. The liver atrophy rate was 14.3% ± 2.3% (PTPE) and 5.4% ± 1.6% (PVL), which was a significant difference (P = 0.0061). To date, the two procedures have been regarded equivalent in effectiveness, however, the results suggest the superiority of PTPE over PVL, and to the best of knowledge, this is the first report to demonstrate such difference.

Terminology

PVL is a procedure to ligate the unilateral portal vein to induce ipsilateral liver atrophy and consequent hypertrophy of the contralateral liver. PTPE is a procedure to occlude the unilateral portal vein by percutaneously injecting embolic agent to induce ipsilateral liver atrophy and consequent hypertrophy of the contralateral liver. TSHP is a surgical strategy adopted for severe metastatic liver cases where it is impossible to remove all malignant lesions in a single procedure. In the first procedure, resectable tumors are removed and after a period of time for liver regeneration, the second procedure is performed to remove the remaining tumors.

Peer review

The current study compared PTPE and unilateral PVL. Using small cohorts, the authors concluded that PTPE is a more effective procedure than PVL as the postoperative liver volume atrophy rate was significantly greater in the PTPE group than the PVL group. This study was novel, well written, technically well conducted, and highly clinically important.

REFERENCES

1 **Kokudo N**, Tada K, Seki M, Ohta H, Azekura K, Ueno M, Ohta K, Yamaguchi T, Matsubara T, Takahashi T, Nakajima T, Muto T, Ikari T, Yanagisawa A, Kato Y. Proliferative activity of intrahepatic colorectal metastases after preoperative hemihepatic portal vein embolization. *Hepatology* 2001; **34**: 267-272

2 **Tanaka H**, Hirohashi K, Kubo S, Shuto T, Higaki I, Kinoshita H. Preoperative portal vein embolization improves prognosis after right hepatectomy for hepatocellular carcinoma in patients with impaired hepatic function. *Br J Surg* 2000; **87**: 879-882

3 **Liu H**, Zhu S. Present status and future perspectives of preoperative portal vein embolization. *Am J Surg* 2009; **197**: 686-690

4 **Hirohashi K**, Tanaka H, Tsukamoto T, Kubo S, Shuto T, Takemura S, Yamamoto T, Kanazawa A, Ogawa M, Osugi H, Kinoshita H. Limitation of portal vein embolization for extension of hepatectomy indication in patients with hepatocellular carcinoma. *Hepatogastroenterology* 2004; **51**: 1084-1087

5 **Hiramatsu K**, Sano T, Nagino M, Nimura Y. Repeat hepatectomy for colonic liver metastasis presenting intrabiliary growth--application of percutaneous transhepatic portal vein embolization for impaired liver. *Hepatogastroenterology* 2007; **54**: 1554-1556

6 **van den Esschert JW**, de Graaf W, van Lienden KP, Busch OR, Heger M, van Delden OM, Gouma DJ, Bennink RJ, Laméris JS, van Gulik TM. Volumetric and functional recovery of the remnant liver after major liver resection with prior portal vein embolization : recovery after PVE and liver resection. *J Gastrointest Surg* 2009; **13**: 1464-1469

7 **Homayounfar K**, Liersch T, Schuetze G, Niessner M, Goralczyk A, Meller J, Langer C, Ghadimi BM, Becker H, Lorf T. Two-stage hepatectomy (R0) with portal vein ligation--towards curing patients with extended bilobular colorectal liver metastases. *Int J Colorectal Dis* 2009; **24**: 409-418

8 **Lin KJ**, Liao CH, Hsiao IT, Yen TC, Chen TC, Jan YY, Chen MF, Yeh TS. Improved hepatocyte function of future liver remnant of cirrhotic rats after portal vein ligation: a bonus other than volume shifting. *Surgery* 2009; **145**: 202-211

9 **Kianmanesh R**, Farges O, Abdalla EK, Sauvanet A, Ruszniewski P, Belghiti J. Right portal vein ligation: a new planned two-step all-surgical approach for complete resection of primary gastrointestinal tumors with multiple bilateral liver metastases. *J Am Coll Surg* 2003; **197**: 164-170

10 **Adam R**, Lucidi V, Bismuth H. Hepatic colorectal metastases: methods of improving resectability. *Surg Clin North Am* 2004; **84**: 659-671

11 **Lygidakis NJ**, Singh G, Bardaxoglou E, Dedemadi G, Sgourakis G, Nestoridis J, Malliotakis A, Pedonomou M, Solomou EK, Safioleas M, Alamani M, Grigorakos L, Merikas EM. Two-stage liver surgery for advanced liver metastasis synchronous with colorectal tumor. *Hepatogastroenterology* 2004; **51**: 413-418

12 **Yamanaka N**, Okamoto E, Kuwata K, Tanaka N. A multiple regression equation for prediction of posthepatectomy liver failure. *Ann Surg* 1984; **200**: 658-663

13 **Yamanaka N**, Okamoto E, Oriyama T, Fujimoto J, Furukawa K, Kawamura E, Tanaka T, Tomoda F. A prediction scoring system to select the surgical treatment of liver cancer. Further refinement based on 10 years of use. *Ann Surg* 1994; **219**: 342-346

14 **Okamoto E**, Kyo A, Yamanaka N, Tanaka N, Kuwata K. Prediction of the safe limits of hepatectomy by combined volumetric and functional measurements in patients with impaired hepatic function. *Surgery* 1984; **95**: 586-592

15 **Rous P**, Larimore LD. Relation of the portal blood to liver maintenance: A demonstration of liver atrophy conditional on compensation. *J Exp Med* 1920; **31**: 609-632

16 **Honjo I**, Suzuki T, Ozawa K, Takasan H, Kitamura O. Ligation of a branch of the portal vein for carcinoma of the liver. *Am J Surg* 1975; **130**: 296-302

17 **Jaeck D**, Oussoultzoglou E, Rosso E, Greget M, Weber JC, Bachellier P. A two-stage hepatectomy procedure combined with portal vein embolization to achieve curative resection for initially unresectable multiple and bilobar colorectal liver metastases. *Ann Surg* 2004; **240**: 1037-1049; discussion 1037-1049

18 **Togo S**, Nagano Y, Masui H, Tanaka K, Miura Y, Morioka D, Endo I, Sekido H, Ike H, Shimada H. Two-stage hepatectomy for multiple bilobular liver metastases from colorectal cancer. *Hepatogastroenterology* 2005; **52**: 913-919

19 **Mueller L**, Hillert C, Möller L, Krupski-Berdien G, Rogiers X, Broering DC. Major hepatectomy for colorectal metastases: is preoperative portal occlusion an oncological risk factor? *Ann Surg Oncol* 2008; **15**: 1908-1917

20 **Capussotti L**, Muratore A, Baracchi F, Lelong B, Ferrero A, Regge D, Delpero JR. Portal vein ligation as an efficient method of increasing the future liver remnant volume in the surgical treatment of colorectal metastases. *Arch Surg* 2008; **143**: 978-982; discussion 982

21 **Makuuchi M**, Thai BL, Takayasu K, Takayama T, Kosuge T, Gunvén P, Yamazaki S, Hasegawa H, Ozaki H. Preoperative portal embolization to increase safety of major hepatectomy for hilar bile duct carcinoma: a preliminary report. *Surgery* 1990; **107**: 521-527

22 **Abulkhir A**, Limongelli P, Healey AJ, Damrah O, Tait P, Jackson J, Habib N, Jiao LR. Preoperative portal vein embolization for major liver resection: a meta-analysis. *Ann Surg* 2008; **247**: 49-57

23 **Palavecino M**, Chun YS, Madoff DC, Zorzi D, Kishi Y, Kaseb AO, Curley SA, Abdalla EK, Vauthey JN. Major hepatic resection for hepatocellular carcinoma with or without portal vein embolization: Perioperative outcome and survival. *Surgery* 2009; **145**: 399-405

24 **de Graaf W**, van den Esschert JW, van Lienden KP, van Gulik TM. Induction of tumor growth after preoperative portal vein embolization: is it a real problem? *Ann Surg Oncol* 2009; **16**: 423-430

25 **Pamecha V**, Levene A, Grillo F, Woodward N, Dhillon A, Davidson BR. Effect of portal vein embolisation on the growth rate of colorectal liver metastases. *Br J Cancer* 2009; **100**: 617-622

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BRIEF ARTICLE

Comparison of sequential and 7-, 10-, 14-d triple therapy for *Helicobacter pylori* infection

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Abstract

AIM: To compare the effectiveness of sequential therapy for *Helicobacter pylori* (*H. pylori*) infection with that of triple therapy of varying durations.

METHODS: The 460 patients enrolled in this study had *H. pylori*-associated gastritis or a gastric or duodenal ulcer. After screening, *H. pylori*-infected patients were randomly assigned to receive either conventional triple therapy for 7, 10 or 14 d, or a new 10-d sequential therapy. Each of the 4 treatment groups included 115 patients. The outcomes of eradication therapy were assessed 4 wk after treatment by the urea breath test and histology.

RESULTS: The overall eradication rate was 81.0%, and eradication rates were 75.7% for 7-d conventional triple therapy, 81.9% for 10-d conventional triple therapy, 84.4% for 14-d conventional triple therapy, and 82.0% for 10-d sequential therapy. Neither intention-to-treat analysis nor per protocol analysis showed significant differences in eradication rates using sequential therapy or the standard triple therapy (P = 0.416 and P = 0.405, respectively).

CONCLUSION: There are no significant differences between 10-d sequential eradication therapy for *H. pylori* and any duration of standard triple treatment in Korean patients.

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Key words: *Helicobacter pylori*; Sequential therapy; Triple therapy; Gastric ulcer; Duodenal ulcer; Gastritis

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Choi HS, Chun HJ, Park SH, Keum B, Seo YS, Kim YS, Jeen YT, Um SH, Lee HS, Kim CD, Ryu HS. Comparison of sequential and 7-, 10-, 14-d triple therapy for *Helicobacter pylori* infection. *World J Gastroenterol* 2012; 18(19): 2377-2382 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i19/2377.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i19.2377

INTRODUCTION

Helicobacter pylori (*H. pylori*), first identified in 1982, is recognized as a risk factor for gastrointestinal ulcers, chronic gastritis, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma. The most widely used eradication therapy at this time combines a proton pump inhibitor (PPI) with 2 antibiotics, amoxicillin and clarithro-

mycin. However, the failure rate of this triple therapy is 10%-20%[1-4] and has increased with the emergence of clarithromycin resistance[5-7]. When first-line triple therapy fails, a quadruple regimen of PPI, bismuth, tetracycline, and metronidazole is usually applied. The failure rate of this second-line therapy (20%-30%) is also high, which may stem from metronidazole resistance[6,8,9].

To address an increase in antibiotic resistance in *H. pylori* in Western countries, a 10-d sequential therapy was proposed. This new regimen includes a PPI plus 1 g of amoxicillin twice daily for the first 5 d followed by a PPI, 500 mg of clarithromycin and 500 mg of tinidazole twice daily for the remaining 5 d. Since Zullo *et al*[10]. first developed this therapy, it has produced higher eradication rates than triple therapy in several studies[11-15]. However, few studies have reported outcomes of sequential therapy in East Asian countries. Since antibiotic-resistant strains differ in geographical distribution, an *H. pylori* eradication therapy may vary in effectiveness between regions[16,17].

Furthermore, no universal evidence-based guidelines have been established for the optimal duration of triple therapy. Some countries prefer a 7-d therapy, and in other countries treatment longer than 7-d is deemed mandatory[1,18,19]. Most previous comparative studies tested the sequential regimen against 7-d or 10-d triple therapy.

We conducted this study to prospectively compare the *H. pylori* eradication rate obtained with a 10-d sequential regimen to the rates achieved with the conventional 7-d, 10-d and 14-d triple regimens in a Korean cohort of *H. pylori*-infected patients.

MATERIALS AND METHODS

Study population

From March 2008 to August 2011, we interviewed and enrolled patients who visited Korea University Anam Hospital with *H. pylori*-positive gastritis or peptic ulcer (gastric and/or duodenal ulcer) identified in gastroduodenoscopy. *H. pylori* infection was detected using a urea breath test, rapid urease test, or histopathological investigation. This was a prospective randomized controlled study.

Four hundred and sixty patients tested positive for *H. pylori* during this time. Study inclusion criteria were *H. pylori*-associated gastritis, or gastric or duodenal ulcer, and age 18 years or older. Criteria for exclusion from the study were: (1) serious kidney disease that required drug dosage adjustment; (2) previous treatment with antibiotics within the past 4 wk; (3) PPI treatment during the previous 8 wk; (4) previous *H. pylori* eradication failure; (5) significant cardiopulmonary, endocrine or hepatic disease, or a hematologic disorder; (6) previous surgery of the upper gastrointestinal tract; (7) history of malignancy; (8) history of drug or alcohol misuse; (9) antiulcer medication treatment within previous 4 wk; (10) systemic glucocorticoid or anticoagulation treatment; (11) severe psychiatric or neurological disease; and (12) current pregnancy or lactation. After screening, participants were randomly assigned to treatment groups using a computer generated list. Patients provided written consent to participate, and the Institutional Review Board of Korea University Hospital approved this study. We conducted the study in agreement with the principles of the Declaration of Helsinki, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use and Guidelines for Good Clinical Practice.

Treatment protocol

Eligible patients who had no documented contraindications were randomly assigned to one of four treatments: (1) standard triple therapy (rabeprazole 20 mg + amoxicillin 1.0 g + clarithromycin 500 mg twice daily) for 7 d; (2) standard triple therapy for 10 d; (3) standard triple therapy for 14 d; and (4) a 10-d sequential treatment (amoxicillin 1.0 g + rabeprazole 20 mg twice daily for the first 5 d, followed by rabeprazole 20 mg + clarithromycin 500 mg + tinidazole 500 mg twice daily for the remaining 5 d).

Confirmation of eradication

At least 4 wk after completion of treatment, a urea breath test and histopathological diagnosis were performed to determine if *H. pylori* had been successfully eradicated. Drug side effects were assessed with a questionnaire.

Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS 18.0 for Windows; SPSS Inc., Chicago, IL, United States). Power calculations to determine sample size based on previously published data[20] showed that 420 patients would be required to detect a treatment difference at the 5% level of significance with a power of 80%. Hence the sample size was set at 460 patients to allow for a possible 10% dropout rate.

Univariate analysis, with age, gender, endoscopic diagnosis, smoking, alcohol habits, and medications (NSAID) as variables, was performed using the χ^2 test. The *H. pylori* eradication rate was determined using intention-to-treat (ITT) and per protocol (PP) analyses using the χ^2 test. $P < 0.05$ was considered statistically significant.

RESULTS

Patient characteristics

The mean age of the 460 patients enrolled in this study was 46.8 years. Two hundred and thirty-nine patients were male and 221 were female. Each of the 4 treatment groups included 115 patients. The groups did not differ significantly in gender, previous disease history, endoscopic diagnosis, smoking, alcohol consumption, NSAID and aspirin use, or use of previous medications (Table 1).

Eradication rates

Twenty-five of the 460 patients did not return to hospital for eradication testing and 8 patients discontinued the

Table 1 Clinical characteristics of patients with *Helicobacter pylori* infection

	7-d eradication	10-d eradication	14-d eradication	10-d sequential eradication	Total	*P* value
Number of patients	115	115	115	115	460	
Gender						0.959
Male	60	58	62	59	239	
Female	55	57	53	56	221	
Endoscopic diagnosis						0.940
GU	48	52	52	48	200	
DU	40	36	32	36	144	
Gastritis as NUD	27	27	31	31	116	
Smokers	40	36	53	44	176	0.117
Alcohol drinkers	42	48	58	56	204	0.123
NSAID/ASA	12	16	19	13	60	0.513

GU: Gastric ulcer; DU: Duodenal ulcer; NUD: Non-ulcer dyspepsia; NSAID: Non-steroidal anti-inflammatory drug; ASA: Acetylsalicylic acid.

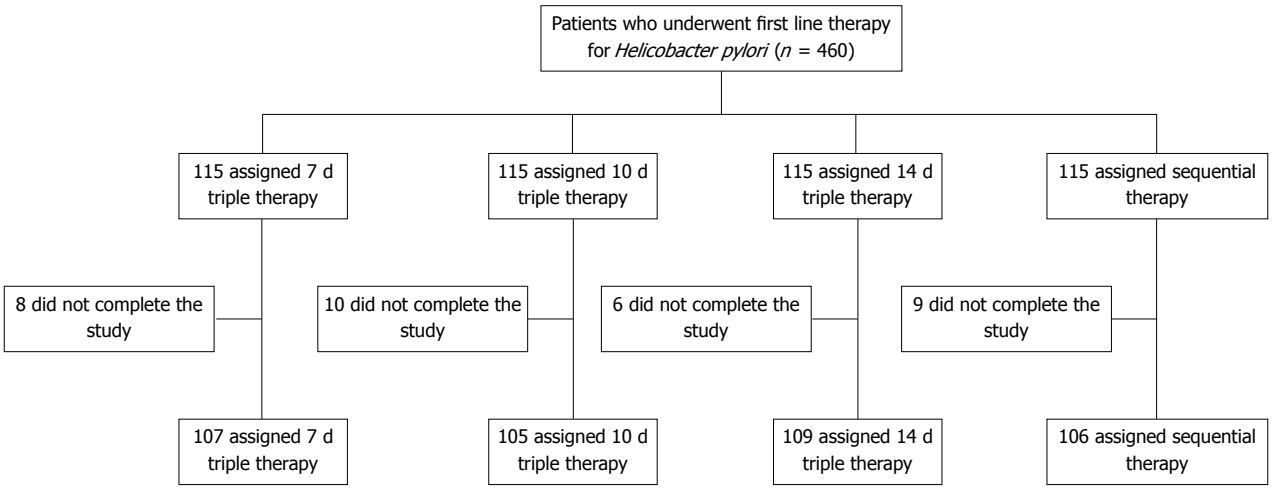

Figure 1 Flow diagram for patients enrolled in the study.

medication due to side effects. Eradication results were confirmed in 427 patients; eradication was confirmed in 408 of the 427 patients by the urea breath test and in 19 patients by histopathological examination for *H. pylori*.

Among the 427 patients, 107 patients received the 7-d conventional triple therapy, 105 received the 10-d conventional triple therapy, 109 received the 14-d conventional triple therapy, and 106 received the 10-d sequential therapy (Figure 1).

The overall eradication rate among the 427 patients was 81.0%. The eradication rates according to group were as follows: 70.4%/75.7% (ITT/PP) for the 7-d conventional triple therapy group, 74.7%/81.9% for the 10-d conventional triple therapy group, 80.0%/84.4% for the 14-d conventional triple therapy group, and 75.6%/82.0% for the 10-d sequential therapy group. Neither the ITT nor the PP analysis showed a significant difference in eradication rates between the new 10-d sequential therapy and the standard triple therapy ($P = 0.416$ and $P = 0.405$, respectively) (Table 2).

Compliance and side-effects

The compliance was greater than 95% for all of the triple conventional groups and the 10-d sequential therapy group. In the latter group, 15 patients showed side effects including problems with taste ($n = 1$), loose stools ($n = 3$), nausea/vomiting ($n = 4$), epigastric discomfort ($n = 3$), abdominal distention ($n = 3$) and itching ($n = 1$) (Table 3). For the triple therapy, reported side effects included problems with taste, loose stools, abdominal discomfort, nausea, vomiting, epigastric discomfort, and itching. Most side effects were not severe (Table 3).

DISCUSSION

We compared the *H. pylori* eradication rates obtained after 10-d sequential therapy with those achieved after 7-, 10- and 14-d conventional triple therapy and found no significant differences.

Triple therapy, which is currently used most widely, combines a PPI with 2 antibiotics such as clarithromycin and amoxicillin[2]. However, resistance to clarithromycin now stands at 13.8%-16.7%[5,21], and recent reports place the rate of *H. pylori* eradication at 74%-83.6%[18,22].

The Maastricht Ⅲ consensus report published in 2005 recommends a quadruple therapy, which includes a PPI, bismuth, tetracycline, and metronidazole, as a second-line eradication therapy[1]. The effectiveness of

Table 2 *Helicobacter pylori* eradication rates according to treatment group

	7-d eradication	10-d eradication	14-d eradication	10-d sequential eradication	Total	*P* value
ITT	70.4%	74.7%	80.0%	75.6%	75.2%	0.416
	81/115	86/115	92/115	87/115	346/460	
PP	75.7%	81.9%	84.4%	82.0%	81.0%	0.405
	81/107	86/105	92/109	87/106	346/427	

ITT: Intention-to-treat; PP: Per protocol.

Table 3 Adverse events

	7-d eradication	10-d eradication	14-d eradication	10-d sequential eradication
Taste alterations	1	1	1	1
Loose stools	3	3	2	3
Abdominal distention	2	3	3	3
Nausea/vomiting	2	4	3	4
Epigastric discomfort	3	2	3	3
Itching	0	1	0	1
Total	11	14	12	15

quadruple therapy is compromised, however, by resistance to metronidazole in *H. pylori*[8,23,24]. The prevalence of metronidazole resistance in Korea has increased from 33.3% in 1994, to 47.7% in 1999, to 66.2% in 2003[25].

In Western countries, increased resistance to triple therapy fosters much discussion. Notably, an increase in clarithromycin resistance found through large-scale studies in several European countries is linked to extensive use of this antibiotic in both children and adults, and to an increasing rate of failure to eradicate *H. pylori* using conventional therapy[20,26-28]. Nevertheless, European guidelines for *H. pylori* treatment specify triple therapy with a PPI, amoxicillin, and clarithromycin or metronidazole for 14 d, or quadruple therapy with a PPI, amoxicillin, clarithromycin, and metronidazole for 10-14 d[1,29].

Zullo *et al*[10] reported a new 10-d sequential therapy for *H. pylori* in 2000. This regimen includes a PPI plus 1 g of amoxicillin for the first 5 d followed by a PPI, 500 mg of clarithromycin, and 500 mg of tinidazole for the remaining 5 d. Some studies report that this regimen yields a higher *H. pylori* eradication rate than triple therapy[11,30,31] and improves the rate in children as well as in adults[32,33]. The mechanisms underlying the effects of the 10-d sequential treatment are not known; however, the early administration of amoxicillin appears to weaken the cell walls of *H. pylori*, reducing resistance to clarithromycin and enhancing the treatment effects[28,34]. The 5-d PPI and amoxicillin regimen may reduce the numbers of *H. pylori* by 50%, which when followed by 5 d of triple therapy, is thought to improve the overall effectiveness of the regimen[35,36]. In addition, the use of 3 or more antibiotics improves the treatment effects[20].

In our patient cohort, the 10-d sequential therapy did not achieve a higher eradication rate than the standard triple therapy. This discrepancy may reflect in part genetic differences in *H. pylori* strains in the East and West[37]. In addition, the high rate of antibiotic resistance in Korea, in particular to metronidazole, may contribute to geographical variations in treatment effectiveness. Factors in addition to location and antibiotic resistance that may affect *H. pylori* eradication include patient age and compliance, gastric acid concentration, individual response to PPI, and differences in prevalence of *H. pylori* Cag A genotype[38]. We did not investigate gastric acid concentration, antibiotic resistance (or minimal inhibitory concentrations), or *H. pylori* genotypes; and as all subjects were recruited at a single center, geographical differences in *H. pylori* sensitivity and in treatment protocol did not emerge in this study.

The optimal treatment duration using triple therapy is not established. A meta-analysis performed by Fuccio *et al*[39] concluded that extending triple therapy beyond 7 d does not significantly improve outcome compared to the standard 7-d regimen. However, individual studies report higher eradication rates using 10 or 14 d of triple therapy as compared to 7 d[40,41]. Guidelines in the United States and Europe favor longer durations of triple therapy[1,19]. Previous comparative studies of the sequential regimen involved the 7-d and 10-d triple therapy. The present study aimed to clarify the relationship of treatment duration to the *H. pylori* eradication rate.

Data from East Asian countries on use of sequential therapy are limited, and further study is needed to determine the optimal duration of treatment for the triple therapy in Asian populations. Several comparative studies of sequential therapy conducted in Korea differed in outcome. Three of these studies produced higher eradication rates for sequential therapy and another failed to detect a significant difference[38,42-45]. This lack of concordance may be related to known regional variations in prevalence of *H. pylori* resistance to antibiotics, especially clarithromycin, in Korea[17].

In conclusion, we detected no significant differences between the 10-d sequential eradication therapy for *H. pylori* and any duration of the standard triple treatment tested (7-, 10- and 14-d regimens) in a group of Korean patients. More research is needed to determine whether this finding also holds true for patients from other East Asian countries.

COMMENTS

Background

Helicobacter pylori (*H. pylori*), first identified in 1982, has been identified as a risk factor for gastrointestinal ulcer, chronic gastritis, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma. To date, the most widely used eradication therapy is administration of a proton pump inhibitor (PPI) and 2 types of antibiotics; amoxicillin and clarithromycin. However, the success rate of these multiple antibiotics to eradicate *H. pylori* has decreased in recent years. An increase in the antibiotic resistance of *H. pylori* has been reported in Western countries, and a 10 d sequential eradication therapy was proposed to address this problem.

Research frontiers

The 10-d sequential therapy includes a PPI plus 1 g amoxicillin for the first 5 d

followed by a PPI, clarithromycin 500 mg, and tinidazole 500 mg for the remaining 5 d. There is no established evidence about the duration of triple therapy. Guidelines for the duration of triple therapy varies according to region. So, a Korean research team evaluated whether *H. pylori* eradication with 10 d of sequential therapy is better than the conventional 7-, 10- and 14-d triple therapy in a Korean cohort.

Applications

The researchers concluded that there was no significant difference between 7-, 10-, 14-d standard triple treatment and 10-d sequential therapy in eradication of *H. pylori* in a Korean cohort of patients. Data from East Asian countries on use of sequential therapy are limited, and further study is needed to determine the optimal duration of treatment for triple therapy in Asian populations.

Terminology

Sequential therapy is a new regimen to eradicate *H. pylori* includes a PPI plus 1 g of amoxicillin twice daily for the first 5 d followed by a PPI, 500 mg of clarithromycin and 500 mg of tinidazole twice daily for the remaining 5 d.

Peer review

This is an interesting study aimed at comparing the eradication rate of a 7, 10 and 14 d triple therapy *vs* sequential therapy in Korean patients. Interestingly, the authors found that the eradication rate provided by sequential therapy is similar to that of standard therapy of the same duration. Therefore, the final message is that the most important factor in the eradication of *H. pylori* is the duration of the therapy instead of the way of administration of antibiotics and PPI.

REFERENCES

1 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781

2 **Fock KM**, Katelaris P, Sugano K, Ang TL, Hunt R, Talley NJ, Lam SK, Xiao SD, Tan HJ, Wu CY, Jung HC, Hoang BH, Kachintorn U, Goh KL, Chiba T, Rani AA. Second Asia-Pacific Consensus Guidelines for Helicobacter pylori infection. *J Gastroenterol Hepatol* 2009; **24**: 1587-1600

3 **Kim BW**, Choi MG, Moon SB, Kim BK, Chae HS, Kim JK, Chung IS, Sun HS, Park DH. Pooled analysis of antibiotic therapy for helicobacter pylori eradication in Korea. *Korean J Gastroenterol* 1999; **34**: 42-49

4 **Sasaki M**, Ogasawara N, Utsumi K, Kawamura N, Kamiya T, Kataoka H, Tanida S, Mizoshita T, Kasugai K, Joh T. Changes in 12-Year First-Line Eradication Rate of Helicobacter pylori Based on Triple Therapy with Proton Pump Inhibitor, Amoxicillin and Clarithromycin. *J Clin Biochem Nutr* 2010; **47**: 53-58

5 **Bang SY**, Han DS, Eun CS, Kim JE, Ahn SB, Sohn JH, Jeon YC, Kang JO. [Changing patterns of antibiotic resistance of Helicobacter pylori in patients with peptic ulcer disease]. *Korean J Gastroenterol* 2007; **50**: 356-362

6 **Mégraud F**, Lehours P. Helicobacter pylori detection and antimicrobial susceptibility testing. *Clin Microbiol Rev* 2007; **20**: 280-322

7 **Vakil N**, Lanza F, Schwartz H, Barth J. Seven-day therapy for Helicobacter pylori in the United States. *Aliment Pharmacol Ther* 2004; **20**: 99-107

8 **Osato MS**, Reddy R, Graham DY. Metronidazole and clarithromycin resistance amongst Helicobacter pylori isolates from a large metropolitan hospital in the United States. *Int J Antimicrob Agents* 1999; **12**: 341-347

9 **Lee H**, Kim JJ. [Impact of metronidazole resistance on eradication rate for Helicobacter pylori]. *Korean J Gastroenterol* 2005; **46**: 142-145

10 **Zullo A**, Rinaldi V, Winn S, Meddi P, Lionetti R, Hassan C, Ripani C, Tomaselli G, Attili AF. A new highly effective short-term therapy schedule for Helicobacter pylori eradication. *Aliment Pharmacol Ther* 2000; **14**: 715-718

11 **Zullo A**, Gatta L, De Francesco V, Hassan C, Ricci C, Bernabucci V, Cavina M, Ierardi E, Morini S, Vaira D. High rate of Helicobacter pylori eradication with sequential therapy in elderly patients with peptic ulcer: a prospective controlled study. *Aliment Pharmacol Ther* 2005; **21**: 1419-1424

12 **Hassan C**, De Francesco V, Zullo A, Scaccianoce G, Piglionica D, Ierardi E, Panella C, Morini S. Sequential treatment for Helicobacter pylori eradication in duodenal ulcer patients: improving the cost of pharmacotherapy. *Aliment Pharmacol Ther* 2003; **18**: 641-646

13 **Vakil N**. New developments in the treatment of Helicobacter pylori: is sequential therapy the answer? *Rev Gastroenterol Disord* 2008; **8**: 217-218

14 **Sánchez-Delgado J**, Calvet X, Bujanda L, Gisbert JP, Titó L, Castro M. Ten-day sequential treatment for Helicobacter pylori eradication in clinical practice. *Am J Gastroenterol* 2008; **103**: 2220-2223

15 **Marshall B**. Sequential therapy for Helicobacter pylori: a worthwhile effort for your patients. *Ann Intern Med* 2008; **148**: 962-963

16 **Kim JY**, Kim NY, Kim SJ, Baik GH, Kim GH, Kim JM, Nam RH, Kim HB, Lee DH, Jung HC, Song IS. [Regional difference of antibiotic resistance of helicobacter pylori strains in Korea]. *Korean J Gastroenterol* 2011; **57**: 221-229

17 **Kim N**, Kim JM, Kim CH, Park YS, Lee DH, Kim JS, Jung HC, Song IS. Institutional difference of antibiotic resistance of Helicobacter pylori strains in Korea. *J Clin Gastroenterol* 2006; **40**: 683-687

18 **Kim BG**, Lee DH, Ye BD, Lee KH, Kim BW, Kim SG, Kim SW, Kim SK, Kim JJ, Kim HY, Park JJ, Park CY, Baik GH, Lee YC, Lee JH, Lee JH, Chun HJ, Hahm KB, Hong SJ, Lee SW, Jung HC. Comparison of 7-day and 14-day proton pump inhibitor-containing triple therapy for Helicobacter pylori eradication: neither treatment duration provides acceptable eradication rate in Korea. *Helicobacter* 2007; **12**: 31-35

19 **Peterson WL**, Fendrick AM, Cave DR, Peura DA, Garabedian-Ruffalo SM, Laine L. Helicobacter pylori-related disease: guidelines for testing and treatment. *Arch Intern Med* 2000; **160**: 1285-1291

20 **Vaira D**, Zullo A, Vakil N, Gatta L, Ricci C, Perna F, Hassan C, Bernabucci V, Tampieri A, Morini S. Sequential therapy versus standard triple-drug therapy for Helicobacter pylori eradication: a randomized trial. *Ann Intern Med* 2007; **146**: 556-563

21 **Kim JM**, Kim JS, Jung HC, Kim N, Song IS. [Antibiotic resistance of Helicobacter pylori isolated from Korean patients in 2003]. *Korean J Gastroenterol* 2004; **44**: 126-135

22 **Lee JH**, Hong SP, Kwon CI, Phyun LH, Lee BS, Song HU, Ko KH, Hwang SG, Park PW, Rim KS, Kim S. [The efficacy of levofloxacin based triple therapy for Helicobacter pylori eradication]. *Korean J Gastroenterol* 2006; **48**: 19-24

23 **Kim N**, Lim SH, Lee KH, Koo MS, Kim JM, Hwang JH, Kim JW, Lee DH, Jung HC, Song IS. [Retreatment of Helicobacter pylori infection with triple therapy after initial treatment failure]. *Korean J Gastroenterol* 2003; **42**: 195-203

24 **Mun GH**, Hahm JS, Ryu KH, Lee OY, Han DS, Yoon BC, Choi HS, Lee MH, Lee CS, Park KN, Kang JO. Metronidazole Resistance and the Eradication of Helicobacter pylori. *Korean J Gastrointest Endosc* 1998; **18**: 847-852

25 **Kim JM**. [Antibiotic resistance of Helicobacter pylori isolated from Korean patients]. *Korean J Gastroenterol* 2006; **47**: 337-349

26 **Koletzko S**, Richy F, Bontems P, Crone J, Kalach N, Monteiro ML, Gottrand F, Celinska-Cedro D, Roma-Giannikou E, Orderda G, Kolacek S, Urruzuno P, Martínez-Gómez MJ, Casswall T, Ashorn M, Bodanszky H, Mégraud F. Prospective multicentre study on antibiotic resistance of Helicobacter pylori strains obtained from children living in Europe. *Gut* 2006; **55**: 1711-1716

27 **Francavilla R**, Lionetti E, Castellaneta S, Margiotta M, Pi-

scitelli D, Lorenzo L, Cavallo L, Ierardi E. Clarithromycin-resistant genotypes and eradication of Helicobacter pylori. *J Pediatr* 2010; **157**: 228-232

28 **De Francesco V**, Margiotta M, Zullo A, Hassan C, Troiani L, Burattini O, Stella F, Di Leo A, Russo F, Marangi S, Monno R, Stoppino V, Morini S, Panella C, Ierardi E. Clarithromycin-resistant genotypes and eradication of Helicobacter pylori. *Ann Intern Med* 2006; **144**: 94-100

29 **Gené E**, Calvet X, Azagra R, Gisbert JP. Triple vs. quadruple therapy for treating Helicobacter pylori infection: a meta-analysis. *Aliment Pharmacol Ther* 2003; **17**: 1137-1143

30 **Jafri NS**, Hornung CA, Howden CW. Meta-analysis: sequential therapy appears superior to standard therapy for Helicobacter pylori infection in patients naive to treatment. *Ann Intern Med* 2008; **148**: 923-931

31 **Tong JL**, Ran ZH, Shen J, Xiao SD. Sequential therapy vs. standard triple therapies for Helicobacter pylori infection: a meta-analysis. *J Clin Pharm Ther* 2009; **34**: 41-53

32 **Gatta L**, Vakil N, Leandro G, Di Mario F, Vaira D. Sequential therapy or triple therapy for Helicobacter pylori infection: systematic review and meta-analysis of randomized controlled trials in adults and children. *Am J Gastroenterol* 2009; **104**: 3069-3079; quiz 1080

33 **Albrecht P**, Kotowska M, Szajewska H. Sequential therapy compared with standard triple therapy for Helicobacter pylori eradication in children: a double-blind, randomized, controlled trial. *J Pediatr* 2011; **159**: 45-49

34 **Webber MA**, Piddock LJ. The importance of efflux pumps in bacterial antibiotic resistance. *J Antimicrob Chemother* 2003; **51**: 9-11

35 **De Francesco V**, Zullo A, Margiotta M, Marangi S, Burattini O, Berloco P, Russo F, Barone M, Di Leo A, Minenna MF, Stoppino V, Morini S, Panella C, Francavilla A, Ierardi E. Sequential treatment for Helicobacter pylori does not share the risk factors of triple therapy failure. *Aliment Pharmacol Ther* 2004; **19**: 407-414

36 **Moshkowitz M**, Konikoff FM, Peled Y, Santo M, Hallak A, Bujanover Y, Tiomny E, Gilat T. High Helicobacter pylori numbers are associated with low eradication rate after triple therapy. *Gut* 1995; **36**: 845-847

37 **Jang S**, Jones KR, Olsen CH, Joo YM, Yoo YJ, Chung IS, Cha JH, Merrell DS. Epidemiological link between gastric disease and polymorphisms in VacA and CagA. *J Clin Microbiol* 2010; **48**: 559-567

38 **Choi WH**, Park DI, Oh SJ, Baek YH, Hong CH, Hong EJ, Song MJ, Park SK, Park JH, Kim HJ, Cho YK, Sohn CI, Jeon WK, Kim BI. [Effectiveness of 10 day-sequential therapy for Helicobacter pylori eradication in Korea]. *Korean J Gastroenterol* 2008; **51**: 280-284

39 **Fuccio L**, Minardi ME, Zagari RM, Grilli D, Magrini N, Bazzoli F. Meta-analysis: duration of first-line proton-pump inhibitor based triple therapy for Helicobacter pylori eradication. *Ann Intern Med* 2007; **147**: 553-562

40 **Calvet X**, García N, López T, Gisbert JP, Gené E, Roque M. A meta-analysis of short versus long therapy with a proton pump inhibitor, clarithromycin and either metronidazole or amoxycillin for treating Helicobacter pylori infection. *Aliment Pharmacol Ther* 2000; **14**: 603-609

41 **Calvet X**, López-Lorente M, Cubells M, Barè M, Gálvez E, Molina E. Two-week dual vs. one-week triple therapy for cure of Helicobacter pylori infection in primary care: a multicentre, randomized trial. *Aliment Pharmacol Ther* 1999; **13**: 781-786

42 **Kwon JH**, Lee DH, Song BJ, Lee JW, Kim JJ, Park YS, Kim N, Jeong SH, Kim JW, Lee SH, Hwang JH, Jung HC, Song IS. Ten-day sequential therapy as first-line treatment for Helicobacter pylori infection in Korea: a retrospective study. *Helicobacter* 2010; **15**: 148-153

43 **Park HG**, Jung MK, Jung JT, Kwon JG, Kim EY, Seo HE, Lee JH, Yang CH, Kim ES, Cho KB, Park KS, Lee SH, Kim KO, Jeon SW. Randomised clinical trial: a comparative study of 10-day sequential therapy with 7-day standard triple therapy for Helicobacter pylori infection in naïve patients. *Aliment Pharmacol Ther* 2012; **35**: 56-65

44 **Kim YS**, Kim SJ, Yoon JH, Suk KT, Kim JB, Kim DJ, Kim DY, Min HJ, Park SH, Shin WG, Kim KH, Kim HY, Baik GH. Randomised clinical trial: the efficacy of a 10-day sequential therapy vs. a 14-day standard proton pump inhibitor-based triple therapy for Helicobacter pylori in Korea. *Aliment Pharmacol Ther* 2011; **34**: 1098-1105

45 **Oh HS**, Lee DH, Seo JY, Cho YR, Kim N, Jeoung SH, Kim JW, Hwang JH, Park YS, Lee SH, Shin CM, Cho HJ, Jung HC, Song IS. Ten-day sequential therapy is more effective than proton pump inhibitor-based therapy in Korea: a prospective, randomized study. *J Gastroenterol Hepatol* 2012; **27**: 504-509

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BRIEF ARTICLE

Hexahydrocurcumin enhances inhibitory effect of 5-fluorouracil on HT-29 human colon cancer cells

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Abstract

AIM: To investigate the ability of hexahydrocurcumin (HHC) to enhance 5-fluorouracil (5-FU) in inhibiting the growth of HT-29 cells by focusing on cyclooxygenase (COX)-2 expression.

METHODS: Antiproliferative effects of HHC and 5-FU, alone and in combination, on growth of HT-29 human colon cancer cells were assessed using 5-diphenyltetrazolium bromide (MTT) reduction assay. In combination treatment, low doses of 5-FU were used combined with various concentrations of HHC to minimize the toxicity and side effects of 5-FU. The therapeutic effects of these drugs on down-regulation of COX-2 mRNA and protein expression were examined using semi-quantitative reverse transcription–polymerase chain reaction (RT-PCR) and Western blotting analysis.

RESULTS: MTT reduction assay indicated that HHC alone markedly decreased the viability of HT-29 human colon cancer cells compared to control. Semi-quantitative RT-PCR analysis indicated that HHC is a selective COX-2 inhibitor. This finding was supported by the observation that HHC significantly down-regulates COX-2 mRNA expression compared to the control (control: 100.05% ± 0.03% *vs* HHC: 61.01% ± 0.35%, $P < 0.05$) but does not alter COX-1 mRNA. In combined treatment, addition of HHC to a low dose of 5-FU exerts a synergistic effect against the growth of HT-29 cells by markedly reducing cell viability to a greater degree than monotherapy. Semi-quantitative RT-PCR indicated that 5-FU at the concentration of 5 μmol/L in combination with HHC at the concentration of 25 μmol/L significantly down-regulates COX-2 mRNA expression when compared with values in cells treated with 5-FU or HHC alone (HHC + 5-FU: 31.93% ± 5.69%, 5-FU: 100.66% ± 4.52% *vs* HHC: 61.01% ± 0.35%, $P < 0.05$).

CONCLUSION: HHC together with 5-FU exerts a synergistic effect and may prove chemotherapeutically useful in treating human colon cancer.

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Key words: Colon cancer; Hexahydrocurcumin; 5-Fluorouracil; Combination treatment; Cyclooxygenase-2; Synergistic effect

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INTRODUCTION

Colorectal cancer is a major cause of cancer death in many countries. 5-Fluorouracil (5-FU) is a chemotherapy drug widely used in treating colorectal cancer. However, the toxicity of 5-FU towards normal cells and resistance to this drug are major barriers to successful cancer chemotherapy. Therefore, the combination of 5-FU and other regimens is often practiced to enhance the efficacy of 5-FU and also to reduce its toxicity. Previous *in vitro* and *in vivo* studies of colon cancer have reported that 5-FU combined with other regimens, such as genistein[1] and geraniol[2], are more effective than 5-FU treatment alone.

Curcumin (diferuloylmethane, Figure 1), the major yellow pigment in turmeric which is obtained from the rhizome of *Curcuma longa L.*[3], has been traditionally used in cooking in India and Southeast Asia. It has also been used in both *in vitro* and *in vivo* studies as a naturally occurring substance to treat a wide variety of cancers, including ovarian[4], lung[5], skin[6] and colon cancer[7-9]. Curcumin specifically inhibits mRNA and protein expression of cyclooxygenase (COX)-2, which is highly expressed in a variety of human cancers[10-12] including colon cancer, but it does not alter the expression of COX-1, the enzyme that maintains normal gastric mucosa and influences kidney function[13,14]. All these results seem to suggest that curcumin might have minimal toxicity and is safe for the treatment of human colon cancer compared with other traditional chemopreventive agents such as non-steroidal anti-inflammatory drugs. Although curcumin is a very important agent in preventing and treating colon cancer, its disadvantages include poor solubility and poor absorption in the gastrointestinal tract. Previous reports have indicated that after oral administration of curcumin, about 60% of the dose was absorbed and 38% remained in the large intestine of rats[15] and it is rapidly decomposed in human blood[16]. Curcumin metabolites were synthesized to solve these problems[17].

Hexahydrocurcumin (HHC, Figure 1) is one of the major metabolites of curcumin. Previous studies revealed that this compound exhibits stronger antioxidant activity than curcumin[18]. Moreover, this compound inhibits the biosynthesis of prostaglandin (PGE_2) in LPS-stimulated macrophages[19]. PGE_2 is a major product of COX-2 enzymes implicated in colorectal carcinogenesis and has been shown to stimulate the growth of human colorectal carcinoma cells. In addition, HHC decreases the level of phorbol ester-induced PGE_2 production in human colonic epithelial cells (HCECs) but weakly inhibits COX-2 protein[20].

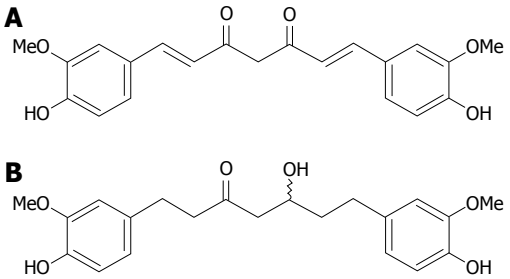

Figure 1 Chemical structures of curcumin (A) and hexahydrocurcumin (B).

All these results suggest that HHC down-regulates COX-2 expression, leading us to hypothesize that the HHC-induced suppression of COX-2 expression may improve the effectiveness of the 5-FU conventional chemotherapy drug. The synergistic effect of using 5-FU in combination with curcumin to inhibit the growth of HT-29 human colon cancer cell line has already been demonstrated[21]. The present study aims to evaluate the ability of HHC to enhance 5-FU in inhibiting the growth of HT-29 human colon cancer cells by focusing on the expression of COX-2.

MATERIALS AND METHODS

Materials

The HT-29 human colon adenocarcinoma cell lines were obtained from the American Type Culture Collection. McCoy's 5A Media Modified medium, fetal bovine serum (FBS), trypsin, penicillin and streptomycin were purchased from GIBCO-BRL (Gaitherburg, MD). 5-FU and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, United States). TRIzol® Reagent was purchased from Invitrogen (Carlsbad, CA). InProm-II™ reverse transcriptase and GoTag® Flexi DNA polymerase were obtained from Promega (Madison, United States). COX-1 monoclonal antibody (1:1000 dilutions) and COX-2 polyclonal antibody (1:500 dilutions) were obtained from Cayman Chemical (Ann Arbor, MI, United States).

Preparation of hexahydrocurcumin

Curcumin was obtained from *Curcuma longa* as described previously[22]. HHC was synthesized from curcumin by catalytic hydrogenation reaction in ethanol for 5 h, with palladium on charcoal as a catalyst. The product was isolated from tetrahydrocurcumin and octahydrocurcumin by silica gel column chromatography, followed by recrystallization with dichloromethane-*n*-hexane to give 45% yield of HHC as a white amorphous solid, m.p. 81-82 ℃. Tetrahydrocurcumin and octahydrocurcumin were obtained in 28% and 11% yields, respectively, after repeated column chromatography. The spectroscopic

(IR, ^{1}H-NMR and mass spectra) data of all the synthesized compounds were identical with those obtained from the previous report[22].

Cell culture and treatment

HT-29 cells were cultured in McCoy's 5A Media Modified containing 10% FBS at 37 ℃ in 5% CO_2 and 90% relative humidity. In all experiments, cells were seeded at 1×10^4 cells per well in 96-well plates for cell viability assay, or 1.5×10^5 cells per well in 6-well plates for RNA extraction and Western blotting analysis. After plating, combinations of 5-FU with HHC were added concurrently to the culture medium. Cells were harvested after various times.

Cell viability assay

HT-29 cells were plated in 96-well plates with 100 μL medium and were exposed to various concentrations of HHC, 5FU or combination of 5FU and HHC for 24 and 48 h. After incubation, cells were treated with 100 μL MTT solution (final concentration, 0.5 mg/mL) at 37 ℃ for an additional 4 h. The formazan crystals were solubilized with 100 μL DMSO and then the absorbance was measured at wavelength 540 nm using a microplate reader (Bio-Tek, Instruments, Winooski, VT, United States).

Analysis of combined effects of 5-FU with HHC

The effectiveness of 5-FU and HHC, alone or in combination, to inhibit growth of HT-29 cells was evaluated by measurement of the combination index (CI)[23], adapted from the method described by Chou *et al*[24]. The fractional inhibitory concentrations were calculated by dividing the concentration of the drug in the combination at IC_{50} by the IC_{50} of the individual drugs. In the following equation, the sum of the dose of 5-FU and the dose of HHC give 50% inhibition of cell growth. CI < 1 indicates a synergistic effect; CI = 1, additive effect; and CI > 1, antagonistic effect. CI = (Dose of 5-FU)/[IC_{50} (5-FU)] + (Dose of HHC)/[IC_{50} (HHC)].

RNA extraction and reverse transcription-polymerase chain reaction

After the HT-29 cells were treated for 24 h, total RNA was extracted using TRIzol reagent (Life Technologies, Carlbad, CA, United States). A volume of 1 μg total RNA from each sample was subjected to reverse transcription using ImProm-II™ Reverse Transcription system (Promega Madison, WI, United States). PCR amplification was performed in a reaction volume of 20 μL containing 2 μL of cDNA product and GoTag® DNA polymerase (Promega, United States) using a PerkinElmer 9600 thermal cycler. Amplification of the constitutively-expressed enzyme d-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The oligonucleotide primers used were: human COX-1, 5'-TGCCCAGCTCCTGGCCCGCCGCTT-3' (sense), 5'-GTGCATCAACACAGGCGCCTCTTC-3' (antisense); human COX-2, 5'-TTCAAATGAGATTGTGGAAAAT-3' (sense), 5'-AGATCATCTCTGCCTGAGTATCTT-3' (antisense); GAPDH, 5'-TCCCTCAAGATTGTGAGCAA-3' (sense), and 5'-AAATGAGCCCCAGCCTTCTCC-3' (antisense). The temperature cycling conditions of amplification were as follows: 15 min at 95 ℃, then 25 cycles at 95 ℃ for 1 min, 55 ℃ for 1 min, 72 ℃ for 1 min, and a final extension at 72 ℃ for 10 min. The amplification products were electrophoresed on a 1.0% agarose gel, visualized by ethidium bromide staining, and photographed gel images were scanned using an image analysis system (Gel Doc 1000; Bio-Rad, Hercules, CA, United States). The intensities of specific COX-1 or COX-2 bands were quantitated in relation to GAPDH bands amplified from the same cDNA using Gene Tools analysis software (Syngene, Cambridge, United Kingdom).

Western blotting analysis

Western blotting analysis was performed to measure protein expression of COX-1, COX-2 or β-actin. Cell lysates were prepared by treating the HT-29 cells in RIPA lysis buffer (1 × PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate and 0.1% SDS) for 30 min. The lysates were sonicated twice for 20 s on ice and then were centrifuged at 10 000 × *g* for 10 min to sediment the particulate material. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed under reducing conditions on 8% polyacrylamide gels. The resolved proteins were transferred to PVDF (Millipore, Bedford, MA, United States) and the membranes were incubated with antibodies against human COX-1 or COX-2 (Cayman Chemical Co.) protein in blocking solution. After washing with PBS plus 0.5% Tween-20, the membranes were then incubated with the secondary antibody goat anti-mouse IgG HRP in blocking solution for 2 h at room temperature. After washing, membrane blots were developed using the Immobilon Western (Millipore) chemiluminescence kit and finally exposed to X-ray film.

Statistical analysis

All values are represented as mean ± SE. Statistical comparisons between different treatment results were analyzed by one-way analysis of variance followed by the Dunnett test. The significance was taken when *P*-values were less than 0.05.

RESULTS

Antiproliferative effect of 5-FU, HHC alone and their combination on HT-29 colon cancer cells

After the HT-29 colon cancer cells were exposed to various concentrations of HHC or 5-FU alone for 24 and 48 h, HHC significantly decreased the viability of HT-29 colon cancer cells as compared to control (Figure 2A, upper panel). The growth inhibition ability of HHC on HT-29 cells was time- and concentration-dependent. A similar result was also observed in the 5-FU-treated group (Figure 2B, lower panel). The half-maximal inhibitory concentration of HHC and 5-FU for inhibiting the growth of HT-29 colon cancer cells at 24 and 48 h exposure was

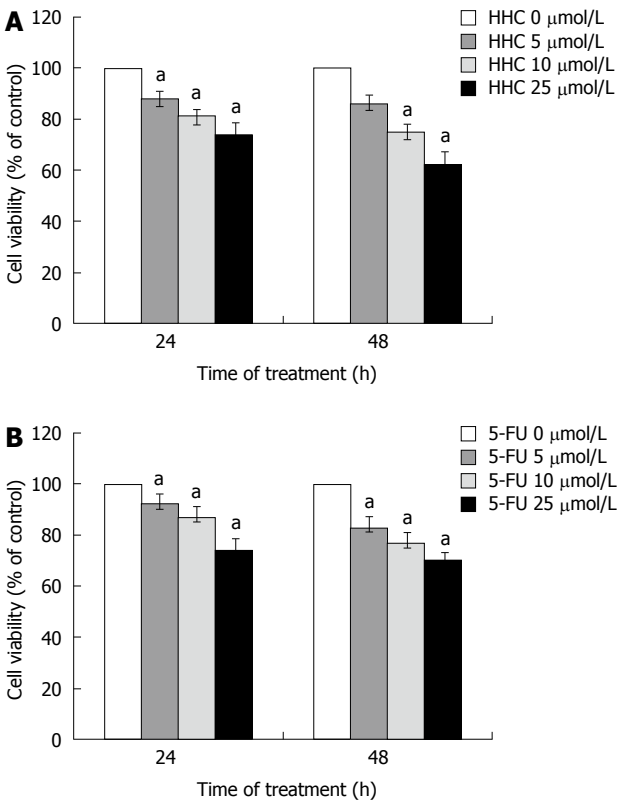

Figure 2 Antiproliferative effect of hexahydrocurcumin and 5-fluorouracil on HT-29 human colon cancer cells. HT-29 cells were exposed to different concentrations of 5-fluorouracil (5-FU) (A) and hexahydrocurcumin (HHC) (B) for 24-48 h and then cell viability was measured by 5-diphenyltetrazolium bromide assay. 5-FU or HHC significantly decreased cell viability concentration-dependently. Data are expressed as mean ± SE. $^aP < 0.05$ *vs* control ($n = 3$).

represented by IC_{50}. The respective IC_{50} values for 24 and 48 h of HHC exposure were 77.05 ± 1.53 and 56.95 ± 2.75, and for 5-FU were 39.13 ± 2.32 and 38 ± 2.21.

To determine the antiproliferative effect of HHC combined with 5-FU, the HT-29 colon cancer cells were exposed to 5-FU at 5 or 25 μmol/L in combination with HHC at 5 or 25 μmol/L concurrently for 24 h and 48 h and then evaluated by MTT assay. After 24 and 48 h of exposure to two doses of HHC (5 and 25 μmol/L) and high dose of 5-FU (25 μmol/L), marked inhibition of the growth of HT-29 colon cancer cells was observed when compared with those treated with 5-FU or HHC alone. Furthermore, exposure to low dose of 5-FU (5 μmol/L) together with high dose of HHC (25 μmol/L) for 24 and 48 h markedly inhibited the viability of HT-29 colon cancer cells when compared with those treated with HHC or 5-FU alone (Figure 3). It was noteworthy that the inhibitory effect of HHC at 25 μmol/L with a low dose of 5-FU was not different from its effect in combination with a high dose of 5-FU. These results indicated that a low dose of 5-FU could effectively be used in combination with high dose of HHC to inhibit the growth of HT-29 colon cancer cells. In addition, the interactions of these two drugs at 25 μmol/L HHC and 5 μmol/L 5-FU for 24 and 48 h were evaluated by the CI at the IC_{50} level as described in materials and methods. The combination of 25 μmol/L HHC with 5 μmol/L 5-FU for 24 and 48 h resulted in a synergistic effect (CI < 1) on the inhibition of the growth of HT-29 colon cancer cells when CI = 0.46 ± 0.01 and 0.57 ± 0.02, respectively.

Combination therapy down-regulation of COX-2 mRNA expression

The MTT results showed that a low dose of 5-FU (5 μmol/L) together with HHC at 25 μmol/L decreased the viability of HT-29 colon cancer cells to a markedly greater extent than 5-FU or HHC exposure alone. Moreover, this treatment showed the quantitative synergistic inhibitory effect; therefore, this combination was chosen to further investigate the effects on COX-2 mRNA expression in HT-29 cells. In this study, we observed that 5-FU alone did not decrease the COX-2 mRNA. However, 5-FU combined with HHC markedly reduced the COX-2 expression compared to HHC or 5-FU alone ($P < 0.05$) (Figure 4). Furthermore, we also observed the combination's effects on the expression of COX-1 mRNA. The result indicated that the level of COX-1 was not altered by treatment with 5-FU or HHC alone, or their combination.

Inhibition of COX-2 protein by the combination of 5-FU with HHC

To investigate the combination's effect on the level of COX-2 protein, HT-29 colon cancer cells were treated with 5-FU (5 μmol/L) together with HHC (25 μmol/L). The results are shown in Figure 5. It is evident that the combination treatment of 5-FU and HHC reduced the COX-2 protein level to a markedly greater degree than did 5-FU or HHC alone ($P < 0.05$). We further examined the combination's effect on the level of COX-1 protein. The results show that 5-FU alone, HHC alone or 5-FU together with HHC did not alter the level of COX-1 protein.

DISCUSSION

The toxicity and the resistance to 5-FU have been major obstacles in successful colorectal cancer chemotherapy. In addition, COX-2 up-regulation is directly involved in colorectal carcinogenesis[25,26]. Therefore, a combined treatment of 5-FU with non-toxic agents that can inhibit COX-2 activity might be useful for treating colon carcinogenesis.

Curcumin, a specific COX-2 inhibitor, is usually used in combination with traditional chemotherapy or other regimens both *in vitro*, *in vivo* and also in clinical studies of colon cancer. Many reports have indicated that curcumin enhanced the cytotoxic effect of celecoxib in several human colon cancer cell lines[27]. This effect has also been observed in colorectal cancer models[28]. Moreover, curcumin enhances the cytotoxic effect of 5-FU and decreases the COX-2 protein expression better than single agent treatment[21]. However, several studies over the past three decades have reported that curcumin exhibits

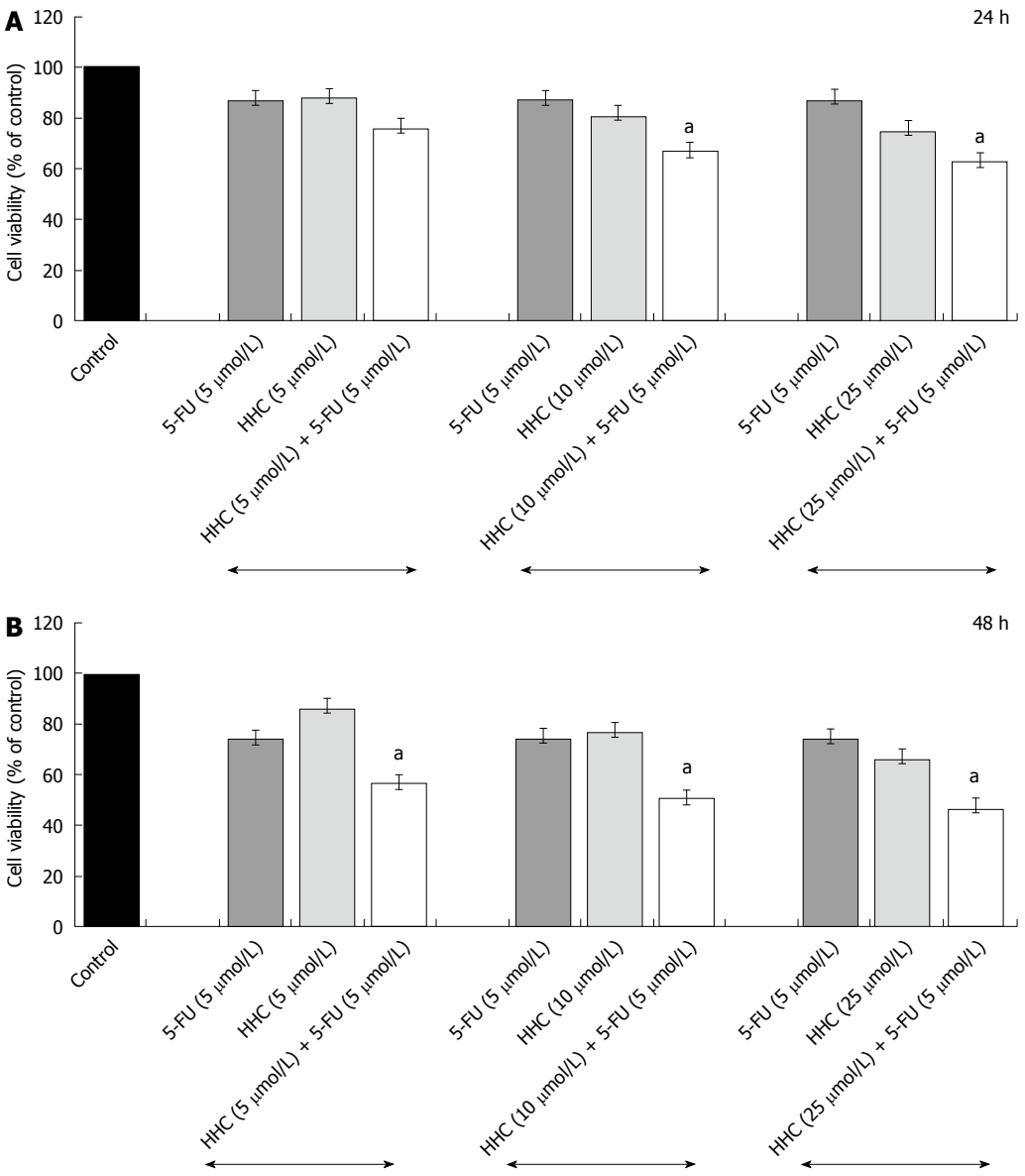

Figure 3 Combination effects of 5-fluorouracil and hexahydrocurcumin on proliferation of HT-29 colon cancer cells. The HT-29 cells were exposed to 5-fluorouracil (5-FU) (5 and 25 μmol/L), hexahydrocurcumin (HHC) (5 and 25 μmol/L) and their combination for 24 h (A) and 48 h (B) and then harvested for 5-diphenyltetrazolium bromide assay. Combination treatment of 5-FU and HHC significantly decreased cell viability compared to the 5-FU or HHC monotherapy. Data are expressed as mean ± SE. $^{a}P < 0.05$ *vs* 5-FU or HHC monotherapy ($n = 3$).

poor bioavailability due to poor absorption, low serum levels and rapid metabolism. Therefore, the pharmacological activity of curcumin may be mediated in part by its metabolites[29,30].

In the present study, we investigated the anti-carcinogenic effects of HHC on growth of HT-29 human colon cancer cells. We found that HHC was sufficient for inhibiting the growth of human colon cancer cells by decreasing the viability of HT-29 cells through down-regulation of COX-2 mRNA and protein expression. Moreover, HHC did not alter COX-1 mRNA and protein expression. These results agreed with previous study, which suggested that HHC could sensitize the cancer cells to chemotherapeutic drugs by decreasing phorbol ester-induced PGE_2 production in HCECs[20]. PGE_2 is a major product of COX-2 enzymes. Moreover, it has been demonstrated that HHC exhibited higher antioxidant activity than its curcumin progenitor[18] and it has been suggested that the hydrogenation at the conjugated double bonds of the central seven-carbon chain and a keto group of the β-diketone of curcumin to HHC markedly enhances antioxidant activity.

Based on these results, it is reasonable to assume that HHC is a safe and effective agent for the treatment of colon cancer, and that this compound is more stable than curcumin. The present investigation deals with the study of the combination effect of HHC with 5-FU chemotherapy drug. Our results indicated that HHC in combination with even a low concentration of 5-FU (5 μmol/L) was sufficient to decrease the viability of HT-29 colon

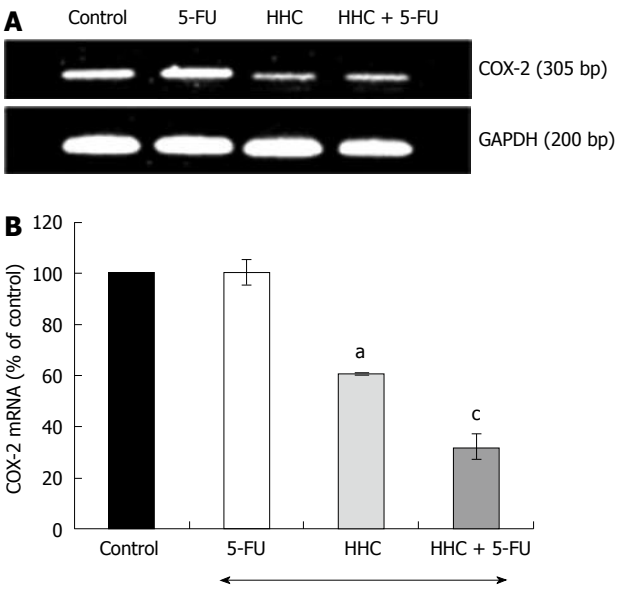

Figure 4 Combination effects of hexahydrocurcumin and 5-fluorouracil on cyclooxygenase-2 mRNA expression in HT-29 colon cancer cells. The HT-29 cells were exposed to 5-fluorouracil (5-FU) (5 μmol/L), hexahydrocurcumin (HHC) (25 μmol/L) and their combination for 24 h. Cells from different experimental groups were subjected to semi-quantitative reverse transcription polymerase chain reaction (RT-PCR). A: RT-PCR showed that the expression of cyclooxygenase (COX)-2 (305 bp) was significantly decreased in HHC or combination of HHC and 5-FU-treated group; B: The expression of COX-2 was determined by normalizing the band intensity of COX-2 with glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Values are expressed as percentage of control (mean ± SE), $^{a}P < 0.05$ *vs* control; $^{c}P < 0.05$ *vs* 5-FU or HHC monotherapy ($n = 3$).

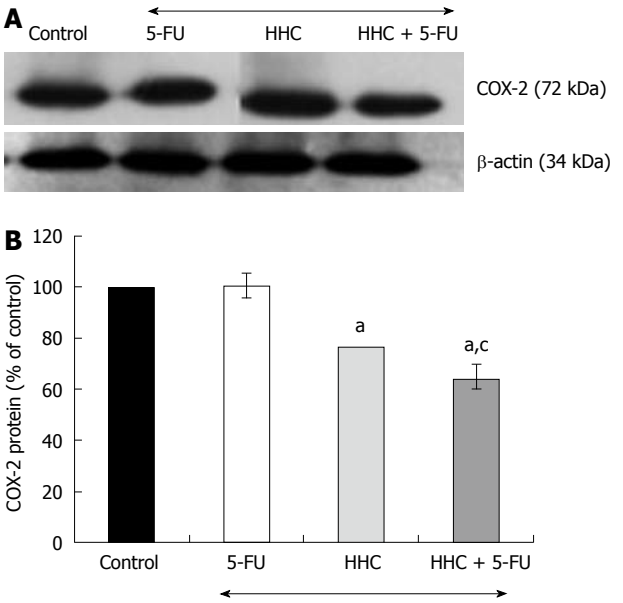

Figure 5 Combination effects of hexahydrocurcumin and 5-fluorouracil on cyclooxygenase-2 protein expression in HT-29 colon cancer cells. A: Western blotting with polyclonal anti-cyclooxygenase (COX) 1 (70 kDa) and anti-COX2 (72 kDa), and anti-β actin (43 kDa) antibodies; B: Fold changes of proteins as represented below, each band was measured by Lab Image software. Combination treatment of 5-fluorouracil (5-FU) and hexahydrocurcumin (HHC) significantly decreased the expression of COX-2 protein with no effect on the expression of COX-1 protein. Values are expressed as percentage of control (mean ± SE), $^{a}P < 0.05$ *vs* control; $^{c}P < 0.05$ *vs* 5-FU or HHC monotherapy ($n = 3$).

cancer cells. Moreover, this combination showed a synergistic effect on antiproliferation against colon cancer cells (CI < 1) and down-regulated the expression of COX-2 mRNA and protein, compared to either 5-FU or HHC monotherapy. Based on these results, we suggest that the addition of HHC could enhance the inhibiting effects of 5-FU on the growth of HT-29 human colon cancer cells. Moreover, the combination of these drugs that are different in their modes of action could prove much safer and more effective than cancer monotherapy[27]. In addition, this combination did not alter the COX-1 mRNA and protein levels. It is thus reasonable to assume that this therapeutic approach may have no toxic effect and enables the use of 5-FU chemotherapy drug at a lower and safer concentration, which would be highly desirable for long-term treatment of colon cancer.

In conclusion, this study illustrates that HHC is a specific COX-2 inhibitor that plays an important role in carcinogenesis. It has been demonstrated for the first time in this study that the addition of HHC to 5-FU standard chemotherapy in HT-29 human colon cancer cells can enhance the growth inhibition and down-regulation of COX-2. Thus, the combination of these two drugs should have a great potential to be used to treat human colon cancer.

COMMENTS

Background

Colorectal cancer is a major cause of cancer death in many countries. The toxicity of 5-fluorouracil (5-FU) chemotherapeutic drug for normal cells and resistance to this drug are major barriers to successful cancer chemotherapy.

Research frontiers

Combining 5-FU with other regimens and agents, such as curcumin, is a strategy to enhance the efficacy of 5-FU and also to reduce its toxicity. Curcumin down-regulates cyclooxygenase (COX)-2, which is highly expressed in human colon cancer, but it does not alter the level of COX-1, a housekeeping enzyme. However, curcumin is only slightly absorbed in the gastrointestinal tract due to its poor solubility in water. Therefore, hexahydrocurcumin (HHC), one of the major curcumin metabolites, was synthesized to solve these problems.

Innovations and breakthroughs

This report has highlighted the importance of HHC which could improve the effectiveness of the 5-FU chemotherapeutic drug. In this study, the authors demonstrate that HHC is a specific COX-2 inhibitor. HHC down-regulates COX-2 expression, but it does not alter the level of COX-1. This is the first study to report the anti-carcinogenic effects of HHC on growth of human colon cancer cells. Furthermore, HHC has ability to enhance 5-FU in inhibiting the growth of HT-29 human colon cancer cells and down-regulates the expression of COX-2 mRNA and protein.

Applications

This report illustrates the action of HHC combined with 5-FU, and this study may represent a future strategy to provide much safer and more effective treatment for patients with colon cancer.

Terminology

HHC is one of the major curcumin metabolites. Curcumin is the major yellow pigment in turmeric which is obtained from the rhizome of *Curcuma longa* L, traditionally used in cooking in India and Southeast Asia.

Peer review

This is a good descriptive study in which authors investigate ability of HHC to enhance 5-FU in inhibiting the growth of HT-29 cells by focusing on COX-2 expression. The results are interesting and suggest that HHC together with 5-FU exerted a synergistic effect and may prove chemotherapeutically useful in treating human colon cancer.

REFERENCES

1 **Hwang JT**, Ha J, Park OJ. Combination of 5-fluorouracil and genistein induces apoptosis synergistically in chemoresistant cancer cells through the modulation of AMPK and COX-2 signaling pathways. *Biochem Biophys Res Commun* 2005; **332**: 433-440

2 **Carnesecchi S**, Bras-Gonçalves R, Bradaia A, Zeisel M, Gossé F, Poupon MF, Raul F. Geraniol, a component of plant essential oils, modulates DNA synthesis and potentiates 5-fluorouracil efficacy on human colon tumor xenografts. *Cancer Lett* 2004; **215**: 53-59

3 **Chuang SE**, Kuo ML, Hsu CH, Chen CR, Lin JK, Lai GM, Hsieh CY, Cheng AL. Curcumin-containing diet inhibits diethylnitrosamine-induced murine hepatocarcinogenesis. *Carcinogenesis* 2000; **21**: 331-335

4 **Wahl H**, Tan L, Griffith K, Choi M, Liu JR. Curcumin enhances Apo2L/TRAIL-induced apoptosis in chemoresistant ovarian cancer cells. *Gynecol Oncol* 2007; **105**: 104-112

5 **Chen YS**, Ho CC, Cheng KC, Tyan YS, Hung CF, Tan TW, Chung JG. Curcumin inhibited the arylamines N-acetyltransferase activity, gene expression and DNA adduct formation in human lung cancer cells (A549). *Toxicol In Vitro* 2003; **17**: 323-333

6 **Kakar SS**, Roy D. Curcumin inhibits TPA induced expression of c-fos, c-jun and c-myc proto-oncogenes messenger RNAs in mouse skin. *Cancer Lett* 1994; **87**: 85-89

7 **Chauhan DP**. Chemotherapeutic potential of curcumin for colorectal cancer. *Curr Pharm Des* 2002; **8**: 1695-1706

8 **Hanif R**, Qiao L, Shiff SJ, Rigas B. Curcumin, a natural plant phenolic food additive, inhibits cell proliferation and induces cell cycle changes in colon adenocarcinoma cell lines by a prostaglandin-independent pathway. *J Lab Clin Med* 1997; **130**: 576-584

9 **Kawamori T**, Lubet R, Steele VE, Kelloff GJ, Kaskey RB, Rao CV, Reddy BS. Chemopreventive effect of curcumin, a naturally occurring anti-inflammatory agent, during the promotion/progression stages of colon cancer. *Cancer Res* 1999; **59**: 597-601

10 **Lee KW**, Kim JH, Lee HJ, Surh YJ. Curcumin inhibits phorbol ester-induced up-regulation of cyclooxygenase-2 and matrix metalloproteinase-9 by blocking ERK1/2 phosphorylation and NF-kappaB transcriptional activity in MCF10A human breast epithelial cells. *Antioxid Redox Signal* 2005; **7**: 1612-1620

11 **Lev-Ari S**, Vexler A, Starr A, Ashkenazy-Voghera M, Greif J, Aderka D, Ben-Yosef R. Curcumin augments gemcitabine cytotoxic effect on pancreatic adenocarcinoma cell lines. *Cancer Invest* 2007; **25**: 411-418

12 **Zhang F**, Altorki NK, Mestre JR, Subbaramaiah K, Dannenberg AJ. Curcumin inhibits cyclooxygenase-2 transcription in bile acid- and phorbol ester-treated human gastrointestinal epithelial cells. *Carcinogenesis* 1999; **20**: 445-451

13 **Goel A**, Boland CR, Chauhan DP. Specific inhibition of cyclooxygenase-2 (COX-2) expression by dietary curcumin in HT-29 human colon cancer cells. *Cancer Lett* 2001; **172**: 111-118

14 **Plummer SM**, Holloway KA, Manson MM, Munks RJ, Kaptein A, Farrow S, Howells L. Inhibition of cyclo-oxygenase 2 expression in colon cells by the chemopreventive agent curcumin involves inhibition of NF-kappaB activation via the NIK/IKK signalling complex. *Oncogene* 1999; **18**: 6013-6020

15 **Ravindranath V**, Chandrasekhara N. Absorption and tissue distribution of curcumin in rats. *Toxicology* 1980; **16**: 259-265

16 **Wang YJ**, Pan MH, Cheng AL, Lin LI, Ho YS, Hsieh CY, Lin JK. Stability of curcumin in buffer solutions and characterization of its degradation products. *J Pharm Biomed Anal* 1997; **15**: 1867-1876

17 **Anand P**, Thomas SG, Kunnumakkara AB, Sundaram C, Harikumar KB, Sung B, Tharakan ST, Misra K, Priyadarsini IK, Rajasekharan KN, Aggarwal BB. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. *Biochem Pharmacol* 2008; **76**: 1590-1611

18 **Somparn P**, Phisalaphong C, Nakornchai S, Unchern S, Morales NP. Comparative antioxidant activities of curcumin and its demethoxy and hydrogenated derivatives. *Biol Pharm Bull* 2007; **30**: 74-78

19 **Shao J**, Lee SB, Guo H, Evers BM, Sheng H. Prostaglandin E2 stimulates the growth of colon cancer cells via induction of amphiregulin. *Cancer Res* 2003; **63**: 5218-5223

20 **Ireson C**, Orr S, Jones DJ, Verschoyle R, Lim CK, Luo JL, Howells L, Plummer S, Jukes R, Williams M, Steward WP, Gescher A. Characterization of metabolites of the chemopreventive agent curcumin in human and rat hepatocytes and in the rat in vivo, and evaluation of their ability to inhibit phorbol ester-induced prostaglandin E2 production. *Cancer Res* 2001; **61**: 1058-1064

21 **Du B**, Jiang L, Xia Q, Zhong L. Synergistic inhibitory effects of curcumin and 5-fluorouracil on the growth of the human colon cancer cell line HT-29. *Chemotherapy* 2006; **52**: 23-28

22 **Changtam C**, de Koning HP, Ibrahim H, Sajid MS, Gould MK, Suksamrarn A. Curcuminoid analogs with potent activity against Trypanosoma and Leishmania species. *Eur J Med Chem* 2010; **45**: 941-956

23 **Chen MF**, Chen LT, Boyce HW. Effect of 5-fluorouracil on methotrexate transport and cytotoxicity in HT29 colon adenocarcinoma cells. *Cancer Lett* 1995; **88**: 133-140

24 **Chou TC**, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. *Adv Enzyme Regul* 1984; **22**: 27-55

25 **Janssen A**, Maier TJ, Schiffmann S, Coste O, Seegel M, Geisslinger G, Grösch S. Evidence of COX-2 independent induction of apoptosis and cell cycle block in human colon carcinoma cells after S- or R-ibuprofen treatment. *Eur J Pharmacol* 2006; **540**: 24-33

26 **Williams CS**, Luongo C, Radhika A, Zhang T, Lamps LW, Nanney LB, Beauchamp RD, DuBois RN. Elevated cyclooxygenase-2 levels in Min mouse adenomas. *Gastroenterology* 1996; **111**: 1134-1140

27 **Lev-Ari S**, Strier L, Kazanov D, Madar-Shapiro L, Dvory-Sobol H, Pinchuk I, Marian B, Lichtenberg D, Arber N. Celecoxib and curcumin synergistically inhibit the growth of colorectal cancer cells. *Clin Cancer Res* 2005; **11**: 6738-6744

28 **Shpitz B**, Giladi N, Sagiv E, Lev-Ari S, Liberman E, Kazanov D, Arber N. Celecoxib and curcumin additively inhibit the growth of colorectal cancer in a rat model. *Digestion* 2006; **74**: 140-144

29 **Shoba G**, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. *Planta Med* 1998; **64**: 353-356

30 **Wahlström B**, Blennow G. A study on the fate of curcumin in the rat. *Acta Pharmacol Toxicol* (Copenh) 1978; 43: 86-92

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BRIEF ARTICLE

A randomized open-label trial of on-demand rabeprazole *vs* ranitidine for patients with non-erosive reflux disease

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Abstract

AIM: To compare the efficacy of the proton-pump inhibitor, rabeprazole, with that of the H_2-receptor antagonist, ranitidine, as on-demand therapy for relieving symptoms associated with non-erosive reflux disease (NERD).

METHODS: This is a single center, prospective, randomized, open-label trial of on-demand therapy with rabeprazole (group A) *vs* ranitidine (group B) for 4 wk. Eighty-three patients who presented to the American University of Beirut Medical Center with persistent gastroesophageal reflux disease (GERD) symptoms and a normal upper gastrointestinal endoscopy were eligible for the study. Patients in group A (n = 44) were allowed a maximum rabeprazole dose of 20 mg twice daily, while those in group B (n = 39) were allowed a maximum ranitidine dose of 300 mg twice daily. Efficacy was assessed by patient evaluation of global symptom relief, scores of the SF-36 quality of life (QoL) questionnaires, total number of pills used, and number of medication-free days.

RESULTS: Among the 83 patients who were enrolled in the study, 76 patients (40 in the rabeprazole group and 36 in the ranitidine group) completed the 4-wk trial. Baseline characteristics were comparable between both groups. After 4 wk, there was no significant difference in the subjective global symptom relief between the rabeprazole and the ranitidine groups (71.4% *vs* 65.4%, respectively; P = 0.9). There were no statistically significant differences between mean cumulative scores of the SF-36 QoL questionnaire for the two study groups (rabeprazole 22.40 ± 27.53 *vs* ranitidine 17.28 ± 37.06; P = 0.582). There was no significant difference in the mean number of pills used (rabeprazole 35.70 ± 29.75 *vs* ranitidine 32.86 ± 26.98; P = 0.66). There was also no statistically significant difference in the mean number of medication-free days between both groups.

CONCLUSION: Rabeprazole has a comparable efficacy compared to ranitidine when given on-demand for the treatment of NERD. Both medications were associated with improved quality of life.

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Key words: Proton-pump inhibitors; H_2-receptor antagonists; Non-erosive reflux disease; Gastroesophageal reflux disease; Quality of life

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Kobeissy AA, Hashash JG, Jamali FR, Skoury AM, Haddad R, El-Samad S, Ladki R, Aswad R, Soweid AM. A randomized open-label trial of on-demand rabeprazole *vs* ranitidine for patients with non-erosive reflux disease. *World J Gastroenterol* 2012; 18(19): 2390-2395 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i19/2390.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i19.2390

INTRODUCTION

Gastroesophageal reflux disease (GERD) is a chronic, remitting-relapsing medical condition that involves the reflux of gastric contents into the esophagus causing a multitude of unpleasant symptoms including heartburn, sore throat, chest pain, cough, and regurgitation. GERD has been shown to have a significant negative impact on the quality of life (QoL) of affected patients and may even impair their daily activities[1]. The prevalence of GERD has markedly increased over the past two decades affecting 20%-40% of the western population[2-5]. This prevalence is predicted to rise further with time[6].

Non-erosive reflux disease (NERD) has been defined by the Vevey NERD Consensus Group as a subcategory of GERD that is characterized by reflux-related symptoms with the absence of esophageal mucosal erosions or breaks at conventional endoscopy and without recent acid-suppressive therapy[7]. About two-thirds of patients with typical GERD symptoms, such as heartburn, belching, cough, nausea, sore throat and voice changes, have no erosive changes on upper gastrointestinal endoscopic evaluation[2]. The complex pathophysiology of NERD and the exact mechanisms by which the associated symptoms are caused remain unclear[7]. It is highly evident, however, that the degree of acidity and duration of esophageal acid exposure play an essential role in NERD symptomatology. These factors are not different from the precipitants of moderate erosive reflux disease (ERD)[7-11].

The majority of GERD patients use acid-suppressive medications to control their symptoms. Unlike ERD, symptoms of NERD are more difficult to control, and tend to have a lower response rate, even to the most potent proton-pump inhibitors (PPIs)[12,13]. Nonetheless, initial management of NERD is similar to that of GERD, and includes the use of a PPI or H_2-receptor antagonist (H_2RA). Around 75% of patients with NERD report a relapse of their symptoms after cessation of the initial therapy. Therefore, long-term management is often needed to maintain symptom control[1]. The preferred maintenance therapeutic strategy is supposed to utilize the least amount of medication. Hence, on-demand therapy should be a reasonable therapeutic mode for managing patients with NERD symptoms. By convention, medications for on-demand therapy should ensure a rapid onset of action in order to give instantaneous relief.

To our knowledge, and after reviewing the literature, no clinical studies have compared a PPI to an H_2RA as on-demand therapy for NERD. The objective of our trial is to compare the efficacy of a PPI, rabeprazole, to that of a standard H_2RA, ranitidine, as an on-demand therapy for treating patients with NERD. We selected rabeprazole among the various currently available PPIs because it results in a faster and a more prompt acid suppression and has the advantage of a first-dose symptom relief[14-16]. H_2RAs including ranitidine are widely used on-demand for the management of GERD symptoms.

MATERIALS AND METHODS

The study was a 4-wk prospective, randomized, single center, open-label trial of on-demand therapy with rabeprazole *vs* ranitidine for patients with NERD. Patients who were considered eligible for enrollment had to be older than 18 years of age, had persistent GERD symptoms (typical or atypical), and a negative upper gastrointestinal endoscopy exam. Exclusion criteria were age below 18 years and older than 75 years, allergy to rabeprazole or ranitidine, any degree of esophagitis or mucosal damage on upper gastrointestinal endoscopy, pregnancy, and any use of PPI or H_2RA within 2 wk of enrollment into the study.

Patients who presented to the American University of Beirut Medical Center with persistent GERD symptoms and a normal upper gastrointestinal endoscopy were considered candidates for the study. After signing a written informed consent, patients were asked to complete a baseline SF-36 QoL questionnaire. They were randomized by an independent investigator using a computer-generated random number table to one of two groups: those to receive rabeprazole (group A) and those to receive ranitidine (group B). Patients in group A were allowed a maximum oral rabeprazole dose of 20 mg (10 mg tablets, twice daily), while those in group B were allowed a maximum oral ranitidine dose of 300 mg (150 mg tablets, twice daily). A research fellow was responsible for contacting patients by phone on a daily basis over the 4-wk study period to assess for the number of pills taken, the need for a rescue medication, as well as the occurrence of any side effects. At the end of the 4 wk, patients were asked about their global symptom relief and were also requested to answer the post-treatment QoL questionnaire.

Primary efficacy endpoints were assessed by the subjective global symptom relief, total number of pills used, number of medication-free days, and the need for rescue medications. Secondary endpoints included the scores of the QoL questionnaires and the occurrence of side effects. The study protocol and informed consent were approved by the Institutional Review Board at the American University of Beirut Medical Center.

Statistical analysis

Analysis of the primary end-point (global symptom relief) was done according to intent-to-treat (ITT) basis.

Table 1 Patient baseline characteristics *n* (%)

	Group A (*n* = 44)	Group B (*n* = 39)	*P* value
Age (mean ± SD) (yr)	45.4 ± 15.2	45.1 ± 15.3	0.916
Gender M:F	16:28	16:23	0.663
Duration of symptoms (yr)	4.68 ± 7.79	3.85 ± 4.21	0.552
Esophageal manifestations	28 (63.6)	29 (74.4)	0.293
Regurgitation and heartburn	21 (47.7)	23 (59.0)	0.306
Epigastric pain	8 (18.2)	9 (23.1)	0.581
Nausea	7 (15.9)	6 (15.4)	0.948
Vomiting	0 (0)	1 (2.6)	0.285
Extra-esophageal manifestations	38 (86.4)	33 (84.6)	0.821
Chest pain	16 (36.4)	10 (25.6)	0.293
Globus	24 (54.5)	16 (41.0)	0.219
Hoarseness	15 (34.1)	10 (25.6)	0.402
Metallic taste	22 (50.0)	16 (41.0)	0.413
Nocturnal cough	17 (38.6)	09 (23.1)	0.127
Dyspnea	21 (47.7)	20 (51.3)	0.746
Sore throat	20 (45.5)	13 (33.3)	0.260
Thick sputum production	24 (54.5)	13 (33.3)	0.052
Mixed symptoms	25 (56.8)	24 (61.5)	0.663

Group A, rabeprazole; Group B, ranitidine. M: Male; F: Female.

The association between the drug group and response for binary measures was assessed using the Fischer's Exact test. For continuous measures, either a two sample *t*-test or Wilcoxon two sample rank sum test were used depending on whether normality held or not. Frequency tables and cross-tabulations were derived in order to depict any associations between the different variables. The paired samples *t*-test and the independent-samples *t*-test were used to compare the QoL score before and after treatment. A *P*-value at or below 0.05 was significant. The data were entered and analyzed using SPSS for Microsoft version 18.0 (SPSS Inc, United States).

Sample size calculation: The sample size was estimated based on an expected response rate of 80% for rabeprazole and 50% for ranitidine. Therefore, we had to include 42 patients in each arm of the study to detect a statistical significance using a power of 80% and a margin of error of 5%.

RESULTS

A total of 83 patients with symptoms consistent with GERD and a negative upper gastrointestinal endoscopy were enrolled in the study. The random assignment of patients into two arms resulted in 44 patients (53%) in group A assigned to receive rabeprazole, and 39 patients (47%) in group B assigned to receive ranitidine. Overall, seven patients dropped out of the study; two patients because of mild medication side effects (both of whom were receiving rabeprazole) and five were lost to follow-up. Seventy-six patients completed the 4-wk trial per-protocol, 40 of whom were assigned to the rabeprazole group and 36 to the ranitidine group. The ITT population consisted of 83 patients.

Baseline characteristics between both groups were comparable (Table 1). The mean age of individuals in group A was 45.43 ± 15.16 years *vs* 45.08 ± 15.29 years for those in group B. There was a slight female predominance in both groups: 63.6% in group A and 59% in group B. Most patients had been symptomatic for more than 1 year, with duration ranging from 3 mo to 20 years. The majority of patients (85.5%) suffered from extra-esophageal manifestations, with globus sensation, metallic taste, shortness of breath, as well as thick sputum production being the commonest among these manifestations. Esophageal manifestations were encountered in approximately 69% of patients.

Table 2 Subjective global symptom assessment *n* (%)

	Better	Same	Worse	Total
Group A	20 (71.4)	7 (25.0)	1 (3.6)	28
Group B	17 (65.4)	8 (30.8)	1 (3.8)	26
Total	37	15	2	54

P = 0.9; Group A, rabeprazole; Group B, ranitidine.

Global symptom improvement was subjectively assessed at the end of the 4-wk treatment phase. Improvement was reported in 71.4% (20 out of 28) of patients in group A and 65.4% (17 out of 26) of patients in group B, while worsening of symptoms was noted in 3.6% and 3.8 % of patients in group A and B, respectively (Table 2). Of the patients in group A, 25% experienced no change in symptoms compared to 30.8% of group B patients (*P* = 0.889). There was no difference in gender distribution between responders and non-responders in either group. However, the mean age of responders to rabeprazole was significantly lower than that of patients who did not respond to this drug (38.90 ± 13.15 years *vs* 53.14 ± 17.68 years; *P* = 0.033). This difference was not noted in patients who received ranitidine.

Regarding the mean number of pills consumed, patients in group A used a mean number of 35.70 ± 29.75 pills of rabeprazole 10 mg, while group B patients consumed a mean number of 32.86 ± 26.98 pills of ranitidine 150 mg, (*P* = 0.66). The mean number of medication-free days was 13.08 ± 9.73 in group A, and 10.78 ± 10.14 in group B (*P* = 0.32).

The SF-36 QoL questionnaire was completed by a total of 65 patients at baseline: 37 in group A (84.1%) and 28 in group B (71.8%). Mean cumulative scores at baseline were comparable between groups A and B, 106.38 ± 30.30 *vs* 104.43 ± 29.82, respectively (*P* = 0.797) (Figure 1). A follow-up SF-36 QoL questionnaire was obtained from 50 patients immediately after the 4-wk treatment phase: 25 were in group A (62.5%) and 25 in group B (69.4%). Mean cumulative scores at follow-up were 135.32 ± 32.19 for group A and 122.76 ± 37.48 for group B (*P* = 0.210). Mean scores of both groups increased significantly (*P* < 0.01 for group A and *P* = 0.028 for group B, compared to respective baseline scores). However, the absolute score differences between baseline and 4 wk for groups A and B were (22.40 ± 27.53

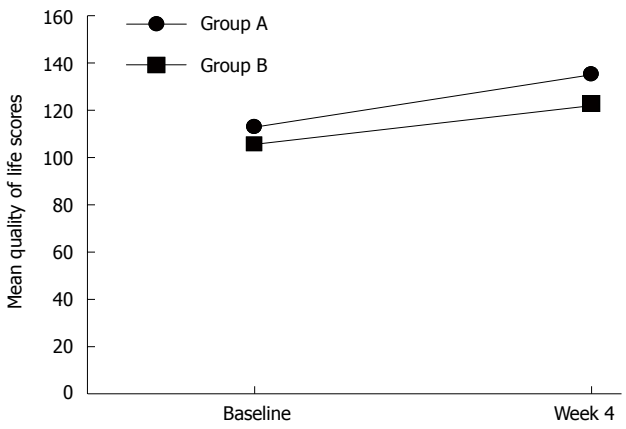

Figure 1 Mean quality of life scores at baseline and immediately after treatment (*n* = 25 at baseline). Group A, rabeprazole; Group B, ranitidine.

vs 17.28 ± 37.06, respectively; $P = 0.582$), indicating that both drugs improve patient QoL to the same extent.

DISCUSSION

The present study compares the efficacy of the PPI rabeprazole to that of the H2RA ranitidine, as an on-demand option in the management of patients with NERD. The impact on QoL was also evaluated as a secondary outcome with both drug regimens.

Analysis of our data showed that rabeprazole is as effective as the routinely and commonly used ranitidine in controlling both the typical and atypical symptoms in patients with NERD. Evidence from several previous studies supports our findings regarding the efficacy of anti-secretory (H2RA and PPI) therapy in patients with NERD[17-24]. However, no head-to-head comparison between any H2RA and PPI had been carried out in the setting of on-demand therapy for NERD.

Our PubMed® search identified a number of clinical trials[14,21-25] that assessed the efficacy of various PPIs (rabeprazole, omeprazole, esomeprazole, lansoprazole) when given as on-demand in patients with NERD. Almost all of these studies were conducted over a period of time ranging from 3 mo to 6 mo and preceded by initial short periods (around 4-8 wk) of daily treatment with the designated PPI to achieve complete symptom resolution. A study by Lind *et al*[21] randomized 424 patients with NERD to one of three groups: omeprazole 20 mg, omeprazole 10 mg, or placebo. After 6 mo of on-demand therapy, it was concluded that omeprazole was effective in the majority of NERD patients. In another placebo-controlled study, Talley *et al*[22] assigned 342 patients to either esomeprazole 20 mg or placebo. On-demand therapy with esomeprazole 20 mg was found suitable for the long-term symptom management of NERD patients. In a separate placebo-controlled trial, Talley *et al*[23] assessed the efficacy of on-demand therapy with esomeprazole 40 mg or 20 mg in patients with NERD and showed that both dosages were superior to placebo in controlling heartburn in those patients. Bytzer *et al*[24] achieved favorable results in a 6-mo trial of on-demand rabeprazole 10 mg in patients with NERD. Five hundred and twenty-three patients with NERD were given 4 wk of rabeprazole 10 mg once daily. The 432 patients who had complete resolution of their symptoms were then randomized for the on-demand phase of the study to two groups: rabeprazole 10 mg and placebo. Symptom relief was significantly better in the rabeprazole group compared to the placebo group.

Rabeprazole was also investigated as on-demand treatment by Ponce *et al*[25] in patients with NERD and low-grade esophagitis. Symptom control was maintained in over 85% of patients during six months of on-demand rabeprazole 20 mg therapy, following a 4-wk daily run-in period with rabeprazole 20 mg per day. During the study period, PPI consumption was found to be low and patient satisfaction with the treatment was high.

Rabeprazole appeared to be ideal for our study given its rapid onset of action and powerful acid suppression[14-16]. Studies involving NERD patients have documented its superiority over placebo. In addition, on-demand use of rabeprazole for the management of NERD incurs the least cost in comparison with other PPIs[26].

H2RAs have been widely used on-demand for patients with GERD. Clinical studies have demonstrated that, when given on-demand, they are superior to placebo in controlling heartburn in this group of patients[18,19,27]. These findings may be extrapolated to NERD patients who constitute the majority of patients with GERD. H2RAs are known to have a rapid onset, but a short duration of action. They suppress acid for approximately 4 to 8 h and produce incomplete inhibition of post-prandial gastric acid secretion. They inhibit acid secretion by up to 70% over a 24-h period. A major disadvantage of using these drugs is the development of tolerance that occurs within two weeks of uninterrupted daily intake[28]. Thus, tolerance would be less concerning if they are to be used on an on-demand basis[29]. PPIs have the advantage over H2RA in controlling both basal and food-stimulated acid secretion producing a longer-lasting acid suppression in addition to the fact that tolerance has not been observed with PPIs.

Our pilot study has a few limitations. One is the open-label nature of the study. Although patients were randomized to different arms, they were aware of the arm they were randomized to. This may have created some bias especially if those patients were previously treated with the same medication class that they were assigned to. The sample size was also relatively small and further investigation based on a larger number of patients is necessary to corroborate our data. Our study duration was short, then again the purpose of our study was not to prove the efficacy of either of the two drugs, but rather to compare them.

The advantages of our study include the fact that it is the first to compare an H2RA to a PPI in the setting of on-demand therapy for NERD. We also showed response to on-demand therapy for both typical and atypical reflux symptoms. Finally, this is a "pure" on-demand study, in

the sense that it was performed without a preceding continuous anti-secretory treatment period.

In conclusion, rabeprazole and ranitidine have been shown to be comparable in efficacy when given on-demand for the treatment of NERD. Both medications were associated with a statistically significant improved quality of life.

COMMENTS

Background

Non-erosive reflux disease (NERD) is the most prevalent of the subcategories of gastroesophageal reflux diseases (GERD). It is a chronic condition with a significant impact on patients' quality of life. On-demand acid-suppressive agents constitute the mainstay in the management of NERD. The most favorable of such agents should be fast-acting, long-lasting, potent, and safe, in order to reach an effective and timely symptom control.

Research frontiers

Acid-suppressive medications differ in terms of power of acid inhibition, onset and duration of action, and drug interactions. Identification of the ideal agent in the context of chronic and on-demand therapy is of utmost interest to patients and researchers alike.

Innovations and breakthroughs

The authors compared the efficacy of the proton-pump inhibitor (PPI) rabeprazole, to that of the H_2-receptor antagonist ranitidine, as on-demand therapy for relieving symptoms associated with NERD. They concluded that both possessed comparable efficacy and were associated with an improved quality of life. Thus, a fast-acting PPI, rabeprazole, is an effective on-demand option for the management of NERD.

Applications

In conditions where an acid-suppressive medication is to be prescribed for a long period of time, recognizing the agent that has the best efficacy and safety profiles would have a prompt and significant clinical impact.

Terminology

NERD is a non-erosive reflux disease, commonly defined as the presence of classic GERD symptoms in the absence of esophageal mucosal injury during upper endoscopy. The majority of patients with GERD fall into the NERD subcategory.

Peer review

The article is novel and interesting. It answers an important research question, which might result in changing clinical practice of NERD management. The design is appropriate, and the manuscript is well written.

REFERENCES

1 **Carlsson R**, Dent J, Watts R, Riley S, Sheikh R, Hatlebakk J, Haug K, de Groot G, van Oudvorst A, Dalväg A, Junghard O, Wiklund I. Gastro-oesophageal reflux disease in primary care: an international study of different treatment strategies with omeprazole. International GORD Study Group. *Eur J Gastroenterol Hepatol* 1998; **10**: 119-124

2 **Fass R**, Fennerty MB, Vakil N. Nonerosive reflux disease--current concepts and dilemmas. *Am J Gastroenterol* 2001; **96**: 303-314

3 **Locke GR**, Talley NJ, Fett SL, Zinsmeister AR, Melton LJ. Prevalence and clinical spectrum of gastroesophageal reflux: a population-based study in Olmsted County, Minnesota. *Gastroenterology* 1997; **112**: 1448-1456

4 **Ronkainen J**, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, Graffner H, Vieth M, Stolte M, Engstrand L, Talley NJ, Agréus L. High prevalence of gastroesophageal reflux symptoms and esophagitis with or without symptoms in the general adult Swedish population: a Kalixanda study report. *Scand J Gastroenterol* 2005; **40**: 275-285

5 **Mishima I**, Adachi K, Arima N, Amano K, Takashima T, Moritani M, Furuta K, Kinoshita Y. Prevalence of endoscopically negative and positive gastroesophageal reflux disease in the Japanese. *Scand J Gastroenterol* 2005; **40**: 1005-1009

6 **El-Serag HB**. Time trends of gastroesophageal reflux disease: a systematic review. *Clin Gastroenterol Hepatol* 2007; **5**: 17-26

7 **Modlin IM**, Hunt RH, Malfertheiner P, Moayyedi P, Quigley EM, Tytgat GN, Tack J, Heading RC, Holtman G, Moss SF. Diagnosis and management of non-erosive reflux disease--the Vevey NERD Consensus Group. *Digestion* 2009; **80**: 74-88

8 **Winter JW**, Heading RC. The nonerosive reflux disease-gastroesophageal reflux disease controversy. *Curr Opin Gastroenterol* 2008; **24**: 509-515

9 **Martínek J**, Benes M, Hucl T, Drastich P, Stirand P, Spicák J. Non-erosive and erosive gastroesophageal reflux diseases: No difference with regard to reflux pattern and motility abnormalities. *Scand J Gastroenterol* 2008; **43**: 794-800

10 **Shapiro M**, Green C, Faybush EM, Esquivel RF, Fass R. The extent of oesophageal acid exposure overlap among the different gastro-oesophageal reflux disease groups. *Aliment Pharmacol Ther* 2006; **23**: 321-329

11 **Martinez SD**, Malagon IB, Garewal HS, Cui H, Fass R. Non-erosive reflux disease (NERD)--acid reflux and symptom patterns. *Aliment Pharmacol Ther* 2003; **17**: 537-545

12 **Dean BB**, Gano AD, Knight K, Ofman JJ, Fass R. Effectiveness of proton pump inhibitors in nonerosive reflux disease. *Clin Gastroenterol Hepatol* 2004; **2**: 656-664

13 **Galmiche JP**. Non-erosive reflux disease and atypical gastro-oesophageal reflux disease manifestations: treatment results. *Drugs* 2006; **66** Suppl 1: 7-13; discussion 29-33

14 **Miner P**, Orr W, Filippone J, Jokubaitis L, Sloan S. Rabeprazole in nonerosive gastroesophageal reflux disease: a randomized placebo-controlled trial. *Am J Gastroenterol* 2002; **97**: 1332-1339

15 **Inamori M**, Togawa J, Takahashi K, Yoneda M, Fujisawa N, Iwasaki T, Ozawa Y, Kikuchi T, Muramatsu K, Chiguchi G, Matsumoto S, Kawamura H, Abe Y, Kirikoshi H, Kobayashi N, Sakaguchi T, Takamura T, Nakajima A, Ueno N, Sekihara H. Comparison of the effect on intragastric pH of a single dose of omeprazole or rabeprazole: which is suitable for on-demand therapy? *J Gastroenterol Hepatol* 2003; **18**: 1034-1038

16 **Pace F**, Pallotta S, Casalini S, Porro GB. A review of rabeprazole in the treatment of acid-related diseases. *Ther Clin Risk Manag* 2007; **3**: 363-379

17 **Johannessen T**, Petersen H, Kristensen P, Fosstvedt D, Kleveland PM, Dybdahl J, Løge I. Cimetidine on-demand in dyspepsia. Experience with randomized controlled single-subject trials. *Scand J Gastroenterol* 1992; **27**: 189-195

18 **Johannessen T**, Kristensen P. On-demand therapy in gastroesophageal reflux disease: a comparison of the early effects of single doses of fast-dissolving famotidine wafers and ranitidine tablets. *Clin Ther* 1997; **19**: 73-81

19 **Galmiche JP**, Shi G, Simon B, Casset-Semanza F, Slama A. On-demand treatment of gastro-oesophageal reflux symptoms: a comparison of ranitidine 75 mg with cimetidine 200 mg or placebo. *Aliment Pharmacol Ther* 1998; **12**: 909-917

20 **Simon TJ**, Berlin RG, Gardner AH, Stauffer LA, Gould AL, Getson AJ. Self-Directed Treatment of Intermittent Heartburn: A Randomized, Multicenter, Double-Blind, Placebo-Controlled Evaluation of Antacid and Low Doses of an H(2)-Receptor Antagonist (Famotidine). *Am J Ther* 1995; **2**: 304-313

21 **Lind T**, Havelund T, Lundell L, Glise H, Lauritsen K, Pedersen SA, Anker-Hansen O, Stubberöd A, Eriksson G, Carlsson R, Junghard O. On demand therapy with omeprazole for the long-term management of patients with heartburn without oesophagitis--a placebo-controlled randomized trial. *Aliment Pharmacol Ther* 1999; **13**: 907-914

22 **Talley NJ**, Venables TL, Green JR, Armstrong D, O'Kane KP, Giaffer M, Bardhan KD, Carlsson RG, Chen S, Hasselgren GS. Esomeprazole 40 mg and 20 mg is efficacious in the long-term management of patients with endoscopy-negative

gastro-oesophageal reflux disease: a placebo-controlled trial of on-demand therapy for 6 months. *Eur J Gastroenterol Hepatol* 2002; **14**: 857-863

23 **Talley NJ**, Lauritsen K, Tunturi-Hihnala H, Lind T, Moum B, Bang C, Schulz T, Omland TM, Delle M, Junghard O. Esomeprazole 20 mg maintains symptom control in endoscopy-negative gastro-oesophageal reflux disease: a controlled trial of 'on-demand' therapy for 6 months. *Aliment Pharmacol Ther* 2001; **15**: 347-354

24 **Bytzer P**, Blum A, De Herdt D, Dubois D. Six-month trial of on-demand rabeprazole 10 mg maintains symptom relief in patients with non-erosive reflux disease. *Aliment Pharmacol Ther* 2004; **20**: 181-188

25 **Ponce J**, Argüello L, Bastida G, Ponce M, Ortiz V, Garrigues V. On-demand therapy with rabeprazole in nonerosive and erosive gastroesophageal reflux disease in clinical practice: effectiveness, health-related quality of life, and patient satisfaction. *Dig Dis Sci* 2004; **49**: 931-936

26 **Hughes DA**, Marchetti M, Colombo G. Cost minimization of on-demand maintenance therapy with proton pump inhibitors in nonerosive gastroesophageal reflux disease. *Expert Rev Pharmacoecon Outcomes Res* 2005; **5**: 29-38

27 **Faaij RA**, Van Gerven JM, Jolivet-Landreau I, Masclee AA, Vendrig EM, Schoemaker RC, Jacobs LD, Cohen AF. Onset of action during on-demand treatment with maalox suspension or low-dose ranitidine for heartburn. *Aliment Pharmacol Ther* 1999; **13**: 1605-1610

28 **Pettit M**. Treatment of gastroesophageal reflux disease. *Pharm World Sci* 2005; **27**: 432-435

29 **Metz DC**, Inadomi JM, Howden CW, van Zanten SJ, Bytzer P. On-demand therapy for gastroesophageal reflux disease. *Am J Gastroenterol* 2007; **102**: 642-653

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BRIEF ARTICLE

Poorly expandable common bile duct with stones on endoscopic retrograde cholangiography

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Abstract

AIM: To describe characteristics of a poorly expandable (PE) common bile duct (CBD) with stones on endoscopic retrograde cholangiography.

METHODS: A PE bile duct was characterized by a rigid and relatively narrowed distal CBD with retrograde dilatation of the non-PE segment. Between 2003 and 2006, endoscopic retrograde cholangiography (ERC) images and chart reviews of 1213 patients with newly diagnosed CBD stones were obtained from the computer database of Therapeutic Endoscopic Center in Chang Gung Memorial Hospital. Patients with characteristic PE bile duct on ERC were identified from the database. Data of the patients as well as the safety and technical success of therapeutic ERC were collected and analyzed retrospectively.

RESULTS: A total of 30 patients with CBD stones and characteristic PE segments were enrolled in this study. The median patient age was 45 years (range, 20 to 92 years); 66.7% of the patients were men. The diameters of the widest non-PE CBD segment, the PE segment, and the largest stone were 14.3 ± 4.9 mm, 5.8 ± 1.6 mm, and 11.2 ± 4.7 mm, respectively. The length of the PE segment was 39.7 ± 15.4 mm (range, 12.3 mm to 70.9 mm). To remove the CBD stone(s) completely, mechanical lithotripsy was required in 25 (83.3%) patients even though the stone size was not as large as were the difficult stones that have been described in the literature. The stone size and stone/PE segment diameter ratio were associated with the need for lithotripsy. Post-ERC complications occurred in 4 cases: pancreatitis in 1, cholangitis in 2, and an impacted Dormia basket with cholangitis in 1. Two (6.7%) of the 28 patients developed recurrent CBD stones at follow-up (50 ± 14 mo) and were successfully managed with therapeutic ERC.

CONCLUSION: Patients with a PE duct frequently require mechanical lithotripsy for stones extraction. To retrieve stones successfully and avoid complications, these patients should be identified during ERC.

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Key words: Common bile duct stone; Difficult stone; Endoscopic retrograde cholangiography; Mechanical lithotripsy

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INTRODUCTION

Endoscopic retrograde cholangiography (ERC) with endoscopic sphincterotomy (ES) and stone extraction are considered standard therapies for the treatment of common bile duct (CBD) stones[1-3]. After ES, 85% to 90% of CBD stones can be removed with a Dormia basket or balloon catheter[4,5]. However, removal of CBD stones can be challenging in certain cases, such as those involving large stones (> 15 mm), stones above strictures, and impacted stones[6]. In cases involving such difficult stones, fragmentation using mechanical lithotripsy or shock wave lithotripsy is required to facilitate stone extraction[6-8]. However, mechanical lithotripsy for extraction of CBD stones can be unsuccessful in the presence of bile duct stricture, when the size of the stone is large, and when the ratio of stone size to bile duct diameter is greater than 1[9-11]. Impaction of an extraction basket and an entrapped stone in the distal CBD may complicate stone clearance in some cases[12-14].

In contrast, anatomic abnormality of the CBD has been considered to be a contributing factor to difficult stones[15]. Kim *et al*[16] recently reported that complete clearance of CBD stones was technically difficult in patients with acute distal CBD angulation (≤ 135 degrees) and a short distal CBD arm (≤ 36 mm). However, the current definition of large (or difficult) CBD stone does not include the factor of distal CBD diameter[17]. In our clinical experience, we have encountered a subgroup of patients whose CBD stones were particularly difficult to extract. Further, in some cases, the extraction basket containing the entrapped stone was impacted in the bile duct if mechanical lithotripsy was not applied. The common characteristic feature of these patients was a relatively narrowed distal CBD on ERC: the distal CBD was almost normal in diameter, but it was poorly expandable (PE), as the upstream CBD was disproportionately dilated. The aim of this study was to describe the clinical and imaging features and to examine the safety and technical success of ERC in patients with both CBD stones and a PE bile duct.

MATERIALS AND METHODS

Description of poorly expandable bile duct

A PE bile duct was characterized by a rigid distal CBD with an almost normal diameter on ERC. The non-PE segment of CBD was often more dilated than the PE segment (Figure 1). The rigid characteristic of the PE segment was usually confirmed by increased resistance when the balloon or basket catheter with the stone was pulled from the non-PE segment across the PE segment.

Patients

During the 4-year period between 2003 and 2006, we retrospectively collected data from 1213 patients with CBD stones from the endoscopic computer database of Change Gung Memorial Hospital, Taipei (China). Endoscopic retrograde cholangiopancreatography (ERCP) images of patients with newly diagnosed CBD stones were reviewed, and 30 (2.5%) of the patients with the characteristics of a PE bile duct were enrolled in this study. The subjects included 20 men and 10 women. The median patient age was 45 years (range, 20 to 92 years). Chart records and the safety and technical success of ERC in these patients were reviewed. None of the patients had a medical history of chronic pancreatitis or biliary stricture prior to ERCP. The clinical diagnoses were obstructive jaundice in 13 cases, acute cholangitis in 10 cases, acute cholecystitis in 3 cases, acute pancreatitis in 1 case, and others in 3 cases. The gallbladder status of the patients was as follows: previously resected in 6 (20%) cases, intact with stone(s) in 23 (76.7%) cases, and intact without stones in 1 (3.3%) case. There were 2 (6.7%) patients with low insertion of the cystic duct and 6 (20%) patients with juxtapapillary diverticulum.

Endoscopic procedures

Endoscopic procedures were performed by endoscopists with an ERCP case volume of at least 2 per week, using a duodenoscope (JF-240 or TJF-240; Olympus). The ES procedure was performed as mentioned in our previous study[18]. After ES, a retrieval balloon catheter (n = 9), Dormia basket (n = 5), or lithotripter (BML-202Q.B or BML-203Q.B, Olympus Corporation, Tokyo, Japan, n = 16) was used to extract the CBD stone(s). When the balloon catheter or Dormia basket failed to retrieve the stone(s), a lithotripter was used to crush and extract the stones. After stone removal, a contrast medium was injected, and the inflated balloon catheter was withdrawn along the CBD to the duodenum to confirm bile duct clearance.

Measurements of parameters on cholangiograms

The maximum transverse diameter of the PE segment, non-PE CBD, and largest stones as well as the length of PE segment and the distal CBD angle were assessed on the cholangiography. The length of the PE segment was defined as the length between the duodenal wall and the tapering end of the non-PE CBD (Figure 1). The distal CBD angle was defined as the angle between the axis of the non-PE CBD and the PE segment (Figure 1A). These factors were measured from the ERC scan, which was obtained under the condition of full contrast injection

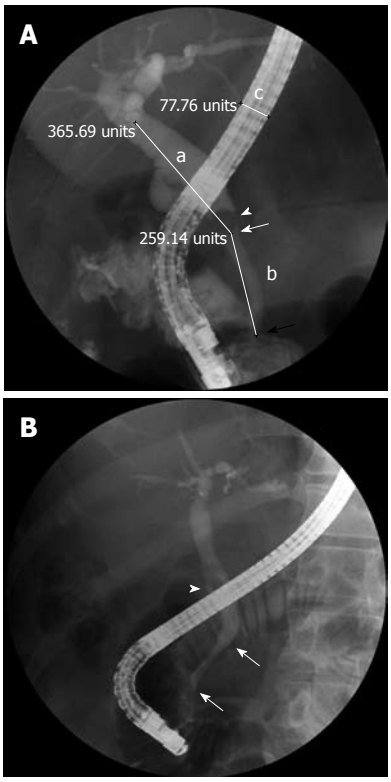

Figure 1 Endoscopic retrograde cholangiogram showing the poorly expandable distal common bile duct (arrows) and the more dilated upstream bile duct. A: The arrowhead indicates the stone. Line a and line b indicate the length of the non-poorly expandable bile duct and the poorly expandable segment. The actual lengths were measured after correction for the magnification with the known diameter of the duodenoscope on the endoscopic retrograde cholangiography (line c). The distal common bile duct angle was defined as the angle formed by line a and line b; B: The stone (arrowhead) is floating in the non-poorly expandable bile duct.

with the patient in a prone position and the duodenoscope in a shortened configuration. The parameters were measured after correction for the magnification using the known diameter of the duodenoscope on the ERC.

Statistical analysis

Data in the text and tables are expressed as the mean ± SD values. The difference in the ratio of the diameters of the non-PE segment to the PE segment and the stone size to the PE segment were compared using the Mann-Whitney *U* test. Further, the difference was compared with the two-sample *t* test for continuous variables and Fisher's exact test for categorical variables. The analyses were performed with SPSS version 17.0 statistical software for Windows. A *P* value of < 0.05 was considered statistically significant.

RESULTS

A total of 30 patients with PE bile ducts who underwent a combined total of 52 ERC procedures were analyzed. The baseline characteristics of these patients are listed in Table 1. The serum total bilirubin level before ERC was 6.5 ± 3.6 mg/dL (range, 0.6 to 15 mg/dL). The maximal diameter of the non-PE CBD was 14.3 ± 4.9 mm (range, 7 mm to 26 mm). The diameter and the length of the PE segment were 5.8 ± 1.6 mm (range, 4 mm to 10 mm) and 39.7 ± 15.4 mm (range, 12.3 mm to 70.9 mm), respectively. The distal CBD angle was 159.3 ± 13.9 degree (range, 130 degree to 175 degree). The number of stones was one in 18 cases, two in 4 cases, three in 7 cases, and five in 1 case. The average diameter of the largest stone from each case was 11.3 ± 4.7 mm (range, 6 mm to 24 mm). Five patients had stones > 15 mm in diameter. Cholesterol stones were found in 16 cases, black stones in 11 cases, and brown stones in 3 cases. The ratio of the maximal diameter of the non-PE CBD to the PE segment was 2.4 (range, 1.4 to 3.5). The ratio of the diameter of the largest stone to the PE segment was 1.8 (range, 0.9 to 4).

In all patients, the CBD stones were eventually retrieved successfully. The number of ERC procedures needed to completely remove the CBD stone(s) was one in 16 (53.3%) cases, two in 8 (26.7%) cases, three in 5 (16.7%) cases, and five in 1 (3.3%) case. The reasons for performing more than one ERC procedure to completely extract the CBD stones were as follows: incomplete clearance of the CBD stones during the first ERC procedure (n = 8), referral from other institutions due to failure to extract the CBD stones (n = 3), recurrent CBD stones due to migration of the cystic duct stones (n = 2), and occurrence of Mirizzi syndrome (n = 1).

Overall, mechanical lithotripsy was performed in 25 (83.3%) patients, including 12 out of the 16 patients undergoing a single session of ERC and 13 out of the 14 patients undergoing multiple (≥ 2) sessions of ERC (4 of the 13 patients did not undergo lithotripsy during the first ERC procedure). Five patients did not require mechanical lithotripsy. Comparative data for the patients with and without lithotripsy for CBD stone clearance are given in Table 1. The factors used for analysis were age; gender; total serum level of bilirubin; stone size, number, and characteristics; history of cholecystectomy; presence of juxtapapillary diverticulum; diameter of the non-PE segment and the PE segment; length of the PE segment; distal CBD angle; and the diameter ratio of the non-PE segment to the PE segment and of the stone to the PE segment. Of the various factors analyzed, the stone size and the ratio of the diameter of the stone to the PE segment were significantly greater in patients who needed lithotripsy (P = 0.04 and 0.02, respectively).

Post-ERC complications occurred in 4 (13.3%) cases, including pancreatitis in 1 (3.3%), cholangitis in 2 (6.7%), and impacted Dormia basket with cholangitis in 1 (3.3%). One patient died of acute myocardial infarction 2 d after ERCP. Twenty-eight patients had long-term follow-up with a mean period of 50 ± 14 mo (range, 29 mo to 80 mo). Two (6.7%) patients developed recurrent CBD stones and were successfully managed with therapeutic ERC.

DISCUSSION

In this study, we describe the ERC findings of patients with concomitant CBD stones and a PE bile duct and the

Table 1 Characteristics of patients with a poorly expandable bile duct and comparisons between the patients with and without mechanical lithotripsy

Parameters	Overall (n = 30)	Need lithotripsy (n = 25)	Without lithotripsy (n = 5)	[1]P value
Age (yr)	47.4 ± 19.4 (20-92)	45.0 ± 19.3 (20-92)	59.0 ± 19.4 (29-76)	0.15
Gender (men) n (%)	20 (66.7)	17 (68)	3 (60)	1
Serum total bilirubin (mg/dL)	6.5 ± 3.6 (0.6-15)	6.7 ± 3.8 (1.1-15)	5.5 ± 3.6 (0.6-10.1)	0.55
History of cholecystectomy n (%)	6 (20)	5 (20)	1 (20)	1
Juxtapapillary diverticulum n (%)	7 (23.3)	5 (20)	2 (40)	0.57
CBD stones				
Size of the largest stone (mm)	11.3 ± 4.7 (6-24)	12.0 ± 4.8 (6-24)	7.2 ± 1.6 (6-10)	0.04
Characteristic (cholesterol/black/brown)	16/11/3	13/9/3	3/2/0	0.72
Number (⩾ 2) n (%)	12 (40)	11 (44)	1 (20)	0.62
Bile duct diameter (mm)				
Non-PE CBD	14.3 ± 4.9 (7-26)	14.8 ± 4.9 (7-26)	11.4 ± 5.0 (7-20)	0.17
PE segment	5.8 ± 1.6 (4-10)	5.9 ± 1.6 (4-10)	5.6 ± 1.8 (4-8)	0.73
Length of the PE segment (mm)	39.7 ± 15.4 (12.3-70.9)	38.0 ± 14.8 (12.3-70.9)	45.1 ± 17.5 (22.5-70.2)	0.44
Distal CBD angle (degree)	159.3 ± 13.9 (130-175)	160 ± 12.1 (135-175)	157 ± 18.9 (130-175)	0.75
Diameter ratio of non-PE segment to PE segment	2.4 ± 0.6 (1.4-3.5)	2.5 ± 0.6 (1.5-3.5)	2.2 ± 0.4 (1.4-2.5)	0.05
Stone to PE segment	1.8 ± 0.7 (0.9-4)	2.1 ± 0.7 (1.2-4)	1.4 ± 0.4 (0.9-1.8)	0.02

CBD: Common bile duct; PE: Poorly expandable. [1]P value was the basis of comparison between patients with and without lithotripsy. A P value of < 0.05 indicated a significant difference.

impact of these findings on the retrieval of the stones. This series showed that 2.5% of patients with CBD stones had PE bile ducts. The mean diameter and length of the PE duct were 5.8 mm and 39.7 mm, respectively. The non-PE CBD was more dilated than the PE segment, with a mean diameter ratio of 2.4. Post-ERC complications occurred in 4 (13.3%) of the patients.

After ES, CBD stones up to 15 mm in diameter can usually be completely extracted with a basket or a retrieval balloon catheter[8,15]. In this study, the average diameter of the largest CBD stones was 11.3 mm, and there were only five patients (16.7%) with stones > 15 mm in diameter. However, mechanical lithotripsy was frequently performed (overall, 83.3%) to clean the CBD stones for patients with a PE bile duct. This rate was much higher compared with the rate reported in the literature (9.4% to 21.7%) for patients with CBD stones who needed lithotripsy[9,10]. In the subsequent analysis, we found that stone size or the ratio of stone size to the PE segment diameter were associated with the need of lithotripsy. Since the stones were relatively small, the major factor associated with the need of lithotripsy was the ratio of stone size to the PE segment diameter. Sharma and Jain reported that 6 of 304 patients (2%) with small CBD stones (7-9 mm) had stones extraction with mechanical lithotripsy due to a narrowed distal CBD (3-4 mm)[19]. These 6 patients might also have the PE bile duct.

The endoscopists frequently chose a lithotripter as the first tool to extract the CBD stones (16/30 or 53.3%), which might be one of the reasons for the high rate of lithotripsy in this study. This practice was adopted because of several cases in our early experience that featured an impaction of the extraction basket and an entrapped stone in the PE segment. Since then, we have often used a lithotripter rather than a Dormia basket to entrap the stone, even when the stone was small. Hence, the authors had only 1 case of biliary-basket impaction in this study. Biliary-basket impaction often occurred upstream of the PE segment. Therefore, we suggest using Conquest through the channel lithotriptor cable for emergency lithotripsy when this complication occurs. In our experience, the Soehendra lithotripter cable is more difficult to pass through the relatively narrowed PE segment.

An impacted CBD stone or a large stone (⩾ 30 mm) poses a high risk of mechanical lithotripsy failure[11]. In this series, CBD stones were successfully removed in all patients for whom mechanical lithotripsy was indicated, although some patients needed more than one endoscopic session. This high success rate was probably due to the small stone size, and there was no case in which the stone was impacted in the bile duct. The non-PE segment was dilated to a greater extent than the PE segment, providing the space for the opening of the lithotripter to capture the stone(s).

Eight (26.7%) of the 30 patients had incomplete clearance of the CBD stones during the first procedure of ERC; therefore, they needed multiple sessions of ERC to clean the stones completely. There might be 2 possible reasons for performing multiple sessions. First, a high proportion of patients underwent mechanical lithotripsy. After lithotripsy, small stone fragments may be left undetected after completion of the ERC procedure with bile duct clearance[20-22]. Second, the distal end of the non-PE segment tapered abruptly at its point of attachment to the PE segment. Similar to the bile duct stricture, some stone fragments might be left in the junction of the non-PE segment and the PE segment during extraction[11].

The cause of PE bile duct remains unknown. Physiologically, the intrapancreatic CBD can be entirely free within the pancreatic capsule, or less commonly, it is partially or completely enclosed by a strip of pancreatic tissue up to 20 mm wide and 3 to 5 mm thick, i.e., the lingula pancreatic[23]. The diameter of the PE segment was 5.8 ±

1.6 mm (range, 4 mm to 10 mm), which was compatible with the reported duct widths for an intrapancreatic portion of CBD[24]. Therefore, the PE bile duct may result from an intrapancreatic CBD that is entirely enclosed by the lingula pancreatis, resulting in the rigid characteristic. However, the length of PE segment had a wide range in this study (12.3 mm to 70.9 mm; mean 39.7 mm), which was not compatible with the length of the lingula pancreatis (range, 10 mm to 25 mm)[23]. Further study may be needed to investigate the nature of the PE bile duct.

The PE bile duct might not increase the risk of the recurrence of CBD stones. Tsuchiya *et al*[20] reported a recurrence rate of 13.2% in patients with CBD stones during a 3-year follow-up study. In a study conducted in Taiwan, the recurrence rate of CBD stones was 18%[25]. In the present series, CBD stone recurred in 7.8% of the patients during the 3-year follow-up period. This rate is no higher than that reported in the literature.

In conclusion, PE bile duct occurred in a small proportion of patients with CBD stones. Lithotripsy or multiple ERC procedures were frequently needed in patients with a PE bile duct. To avoid the complication of biliary-basket impaction, we suggest the use of a lithotripter rather than a Dormia basket to entrap and extract the stone when a PE bile duct is noted on ERC during the initial procedure.

COMMENTS

Background

There were several cases in early experience that featured an impaction of the extraction basket and an entrapped stone in the distal common bile duct (CBD) rather than in the papillary orifice after endoscopic sphincterotomy. Since then, the authors found that the common characteristic of these patients was a poorly expandable (PE) distal CBD on endoscopic retrograde cholangiography (ERC). Therefore, the authors conducted this study to describe the ERC findings of patients with CBD stones and a PE bile duct and evaluate the impact of these findings on the retrieval of the stones.

Research frontiers

CBD stones in the PE bile duct have not been reported in the literature. To the knowledge, this is the first study to describe the clinical and imaging features and to examine the safety and technical success of ERC in patients with both CBD stones and a PE bile duct.

Innovations and breakthroughs

The study showed that the diameters of the PE segment and the widest non-PE CBD segment were 5.8 ± 1.6 mm and 14.3 ± 4.9 mm, respectively. The length of the PE segment was 39.7 ± 15.4 mm (range, 12.3 mm to 70.9 mm). To remove the CBD stone(s) completely, mechanical lithotripsy was required in 83.3% of the patients, even though the stone size was not as large (11.2 ± 4.7 mm). The stone size and stone/PE segment diameter ratio were associated with the need for lithotripsy.

Applications

Patients with CBD stones and a PE duct frequently require mechanical lithotripsy during therapeutic ERC, especially when the ratio of the stone diameter to the PE segment diameter is high. To achieve successful stone retrieval and avoid complications, the authors suggest the use of a lithotripter rather than a Dormia basket to entrap and extract the stone when a PE bile duct is noted on ERC during the initial procedure.

Terminology

A PE bile duct was characterized by a rigid distal CBD with an almost normal diameter on ERC. The non-PE segment of CBD was often more dilated than the PE segment. The rigid characteristic of the PE segment was usually confirmed by increased resistance when the balloon or basket catheter with the stone was pulled from the non-PE segment across the PE segment.

Peer review

The authors have performed a study in which they identified all patients with CBD stones and a poorly expandable bile duct by means of a retrospective search in a computerized database. A total of 30 patients were found and the authors attempted to describe their characteristics. They concluded that the majority of these patients needed mechanical lithotripsy, especially those with a high ratio of stone diameter to the poorly-expandable CBD segment diameter. This a well-written paper and an interesting contribution to the understanding of factors related to difficult CBD stone extraction.

REFERENCES

1 **Williams EJ**, Green J, Beckingham I, Parks R, Martin D, Lombard M. Guidelines on the management of common bile duct stones (CBDS). *Gut* 2008; **57**: 1004-1021

2 **Maple JT**, Ben-Menachem T, Anderson MA, Appalaneni V, Banerjee S, Cash BD, Fisher L, Harrison ME, Fanelli RD, Fukami N, Ikenberry SO, Jain R, Khan K, Krinsky ML, Strohmeyer L, Dominitz JA. The role of endoscopy in the evaluation of suspected choledocholithiasis. *Gastrointest Endosc* 2010; **71**: 1-9

3 **Kawai K**, Akasaka Y, Murakami K, Tada M, Koli Y. Endoscopic sphincterotomy of the ampulla of Vater. *Gastrointest Endosc* 1974; **20**: 148-151

4 **Cotton PB**. Non-operative removal of bile duct stones by duodenoscopic sphincterotomy. *Br J Surg* 1980; **67**: 1-5

5 **Yoo KS**, Lehman GA. Endoscopic management of biliary ductal stones. *Gastroenterol Clin North Am* 2010; **39**: 209-227

6 **Binmoeller KF**, Schafer TW. Endoscopic management of bile duct stones. *J Clin Gastroenterol* 2001; **32**: 106-118

7 **Adler DG**, Conway JD, Farraye FA, Kantsevoy SV, Kaul V, Kethu SR, Kwon RS, Mamula P, Pedrosa MC, Rodriguez SA, Tierney WM. Biliary and pancreatic stone extraction devices. *Gastrointest Endosc* 2009; **70**: 603-609

8 **Muratori R**, Azzaroli F, Buonfiglioli F, Alessandrelli F, Cecinato P, Mazzella G, Roda E. ESWL for difficult bile duct stones: a 15-year single centre experience. *World J Gastroenterol* 2010; **16**: 4159-4163

9 **Garg PK**, Tandon RK, Ahuja V, Makharia GK, Batra Y. Predictors of unsuccessful mechanical lithotripsy and endoscopic clearance of large bile duct stones. *Gastrointest Endosc* 2004; **59**: 601-605

10 **Cipolletta L**, Costamagna G, Bianco MA, Rotondano G, Piscopo R, Mutignani M, Marmo R. Endoscopic mechanical lithotripsy of difficult common bile duct stones. *Br J Surg* 1997; **84**: 1407-1409

11 **Lee SH**, Park JK, Yoon WJ, Lee JK, Ryu JK, Kim YT, Yoon YB. How to predict the outcome of endoscopic mechanical lithotripsy in patients with difficult bile duct stones? *Scand J Gastroenterol* 2007; **42**: 1006-1010

12 **Attila T**, May GR, Kortan P. Nonsurgical management of an impacted mechanical lithotriptor with fractured traction wires: endoscopic intracorporeal electrohydraulic shock wave lithotripsy followed by extra-endoscopic mechanical lithotripsy. *Can J Gastroenterol* 2008; **22**: 699-702

13 **Schreurs WH**, Juttmann JR, Stuifbergen WN, Oostvogel HJ, van Vroonhoven TJ. Management of common bile duct stones: selective endoscopic retrograde cholangiography and endoscopic sphincterotomy: short- and long-term results. *Surg Endosc* 2002; **16**: 1068-1072

14 **Sauter G**, Sackmann M, Holl J, Pauletzki J, Sauerbruch T, Paumgartner G. Dormia baskets impacted in the bile duct: release by extracorporeal shock-wave lithotripsy. *Endoscopy* 1995; **27**: 384-387

15 **Lauri A**, Horton RC, Davidson BR, Burroughs AK, Dooley JS. Endoscopic extraction of bile duct stones: management related to stone size. *Gut* 1993; **34**: 1718-1721

16 **Kim HJ**, Choi HS, Park JH, Park DI, Cho YK, Sohn CI, Jeon WK, Kim BI, Choi SH. Factors influencing the technical difficulty of endoscopic clearance of bile duct stones. *Gastrointest Endosc* 2007; **66**: 1154-1160
17 **Maple JT**, Ikenberry SO, Anderson MA, Appalaneni V, Decker GA, Early D, Evans JA, Fanelli RD, Fisher D, Fisher L, Fukami N, Hwang JH, Jain R, Jue T, Khan K, Krinsky ML, Malpas P, Ben-Menachem T, Sharaf RN, Dominitz JA. The role of endoscopy in the management of choledocholithiasis. *Gastrointest Endosc* 2011; **74**: 731-744
18 **Tsou YK**, Lin CH, Liu NJ, Tang JH, Sung KF, Cheng CL, Lee CS. Treating delayed endoscopic sphincterotomy-induced bleeding: epinephrine injection with or without thermotherapy. *World J Gastroenterol* 2009; **15**: 4823-4828
19 **Sharma SS**, Jain P. Should we redefine large common bile duct stone? *World J Gastroenterol* 2008; **14**: 651-652
20 **Tsuchiya S**, Tsuyuguchi T, Sakai Y, Sugiyama H, Miyagawa K, Fukuda Y, Ando T, Saisho H, Yokosuka O. Clinical utility of intraductal US to decrease early recurrence rate of common bile duct stones after endoscopic papillotomy. *J Gastroenterol Hepatol* 2008; **23**: 1590-1595
21 **Ando T**, Tsuyuguchi T, Okugawa T, Saito M, Ishihara T, Yamaguchi T, Saisho H. Risk factors for recurrent bile duct stones after endoscopic papillotomy. *Gut* 2003; **52**: 116-121
22 **Saito M**, Tsuyuguchi T, Yamaguchi T, Ishihara T, Saisho H. Long-term outcome of endoscopic papillotomy for choledocholithiasis with cholecystolithiasis. *Gastrointest Endosc* 2000; **51**: 540-545
23 Gallbladder and Bile ducts. In: Kremer K, Lierse W, Platzer W, Schreiber HW, Weller S, Steichen FM, editors. Atlas of Operative Surgery. Stuttgart, New York: Georg Thieme Verlag, 1992: 44
24 **Lasser RB**, Silvis SE, Vennes JA. The normal cholangiogram. *Am J Dig Dis* 1978; **23**: 586-590
25 **Lai KH**, Lo GH, Lin CK, Hsu PI, Chan HH, Cheng JS, Wang EM. Do patients with recurrent choledocholithiasis after endoscopic sphincterotomy benefit from regular follow-up? *Gastrointest Endosc* 2002; **55**: 523-526

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BRIEF ARTICLE

Prognosis of HER2 over-expressing gastric cancer patients with liver metastasis

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Abstract

AIM: To study the risk factors for liver metastasis and the prognosis in patients with human epidermal growth factor receptor 2 (HER2) over-expressing gastric cancer (GC).

METHODS: A total of 84 GC patients recruited from the General Hospital of the People's Liberation Army (PLA) between 2003 and 2010 were randomly enrolled in this study. HER2 expression was detected by immunohistochemistry in 84 GC patients with liver metastases. The study group consisted of 66 men and 18 women, with an average age of 54 years (range: 19-74 years). Liver metastasis was diagnosed by magnetic resonance imaging or computed tomography. Patients were followed-up and predictive factors of liver metastasis were evaluated.

RESULTS: The median follow-up period was 47 mo (range: 6-85 mo). The characteristics of 35 (25.7%) patients with HER2 over-expression of liver metastatic GC are presented. HER2 over-expression was detected in 23 out of 49 (46.9%) patients with intestinal GC, and 9 out of 35 (25.7%) patients with diffuse GC. 29 out of 59 (49.2%) patients aged < 60 years were HER2-positive, while 8 out of 25 (32%) patients aged ⩾ 60 were HER2-positive; a significant difference ($P < 0.05$). Univariate analysis (log-rank test) showed that HER2 over-expression, sex, Lauren classification, differentiation and disease-free interval were correlated with poor survival ($P < 0.05$). Survival analysis with a survival curve showed that HER2 over-expression was significantly relevant, with a reduced survival time in GC patients with liver metastases ($P < 0.01$). 2-year survival was not associated with the patient's age. A disease-free survival longer than 12 mo has a significant association with extended overall survival (OS) in GC patients with liver metastases. The median survival time after the diagnosis of liver metastases was 18 mo [95% confidence interval (CI): 9.07-26.94] among HER2 positive GC patients with liver metastases. In comparison, for 49 (69.4%) out of 84 HER2 negative patients with liver metastatic GC, the median survival time was 47 mo (95% CI: 19.37-74.63). In patients with HER2 positive liver metastatic GC, the median OS was significantly shorter than in HER2 negative patients (median, 20.32 mo; 95% CI: 16.51-24.13 *vs* median, 50.14 mo; 95% CI: 37.83-62.45; $P < 0.01$).

CONCLUSION: HER2 over-expressing GC patients with liver metastases have a poor prognosis. Overall survival was significantly lower in HER2 positive patients. HER2-overexpression is correlated with a lower survival rate.

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Key words: Human epidermal growth factor receptor 2;

Overexpression; Gastric cancer; Liver metastasis; Overall survival; Prognosis

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INTRODUCTION

Although the incidence of gastric cancer (GC) has slightly declined in the past several decades, it remains the second leading cause of cancer death worldwide, especially in Asia[1]. GC with human epidermal growth factor receptor 2 (HER2) over-expression accounts for 9%-38% of GC, and HER2 is mainly expressed on the cell membrane. Yan *et al*[2] studied HER2/neu protein expression and gene amplification in gastric carcinoma and their relationship. They recommended that all samples with immunohistochemistry (IHC) as HER2 expression should be analyzed with fluorescence *in situ* hybridization and found that the detection of *HER2* gene amplification can assess the malignant biological behaviors and prognosis of gastric cancer. Zhang *et al*[3] found HER2 over-expression in 18.6% of GC and HER2 located mostly on the membrane, which was consistent with what was previously reported. Yu *et al*[4] examined the expressions of Grb2 and HER2 in normal gastric mucosa, primary gastric cancer, and lymph node metastatic foci in 1143 GC patients by using tissue microarray assay, which suggested the importance of Grb2 and HER2 in GC development and progression. Matsubara *et al*[5] investigated 86 patients who received first-line chemotherapy for advanced GC. Their results showed that patients with high HER2 expression had a longer survival time than those with low HER2 expression. However, there is controversy with regard to the hypothesis that HER2 expression serves as a factor in predicting the prognosis of GC patients[6-10]. In a prospective study of 63 patients with resectable CG (in which follow-up was performed for 40.7 mo), membranous epidermal growth factor receptor (EGFR) was detected by random and double blind assays, and cytosolic HER2 by immunoenzymatic assay. Results showed that high expression of EGFR and HER2 in cancers were associated with a poor outcome in patients with resectable GC[11].

Detection of micrometastatic foci may allow for a more accurate assessment of the prognosis, along with aiding in the selection of candidates for intensive chemotherapy among GC patients. Bone marrow micrometastasis has been shown to influence the prognosis of GC patients[12,13]. Matsunami *et al*[14] used ion mobility spectrometry to improve detection sensitivity in their study, and their results showed that only 30% of patients showed the development of bone marrow micrometastasis. The positive rate was lower than that in Western patients, but similar to those of the Japanese[15]. In the present study, we investigated the prognosis of GC patients with HER2 over-expression in the liver metastatic foci in Chinese patients.

MATERIALS AND METHODS

Patient selection and clinicopathologic parameters

A total of 84 GC patients recruited from the General Hospital of the People's Liberation Army (PLA) between 2003 and 2010 were randomly enrolled in this study. All patients underwent radical dissection and chemotherapy, and/or radiotherapy. The study group consisted of 66 men and 18 women, with an average age of 54 years (range: 19-74 years). GC tissue and adjacent normal tissue were collected for pathologic examination. The detailed pathologic results were obtained from the Department of Pathology of the General Hospital of the PLA.

The histologic type of GC was defined according to Lauren's classification. This classification divides GC into intestinal (in which well-formed tubules were found) and diffuse (in which diffuse tumor infiltration and signet ring cells were noted, but well-formed tubules were absent) types. In our study, 53 patients were pathologically diagnosed with intestinal GC and 31 with diffuse GC.

Patients were followed-up for 6-85 mo. Informed consent was obtained before the study, and the protocol was approved by the Clinical Research Ethics Committee of the General Hospital of the PLA.

Immunohistochemistry

Formalin-fixed and paraffin-embedded sections (5 μm) were deparaffinized in xylene, rehydrated through an ethanol series, and treated for 10 min in a microwave oven at 98 ℃ for antigen retrieval. Sections were then blocked in 3% hydrogen peroxidase, followed by incubation with a protein-blocking agent. Sections were treated with fetal bovine serum (10%), with or without the HER2 antibody (1:100), for 30 min at 37 ℃ then counterstained with hematoxylin and mounted. Omission of the primary antibody was used as a negative control for tissue sections. Anti-HER2 monoclonal antibody (Dako Herceptin Test kit; Dako, Glostrup, Denmark), and Dako EnVisionTM Kit (Dakocytomation, Dako) were used for IHC. The sections were examined microscopically and interpreted in a blinded fashion by two pathologists. HER2 protein expression on the cell membrane was scored according to the following criteria: 0: No staining or < 10% of tumor cells; 1+: Faint/barely perceptible partial staining in > 10% of tumor cells; 2+: Weak to moderate staining of the entire membrane or cytoplasm in > 10% of tumor cells; 3+: Strong staining in > 10% of tumor cells. Tumors with scores of 0 and 1+ were evaluated as negative while those with scores 2+ and 3+ were positive.

Table 1 Human epidermal growth factor receptor 2 expression in the liver metastatic foci in gastric cancer patients

Parameter	Patient (*n*)	HER2 + patients (*n*)	Ratio (%)	*P* value
Age (yr)				
< 60	59	29	49.2	< 0.05
⩾ 60	25	8	32	
Gender				
Male	66	28	42.4	> 0.05
Female	18	7	38.9	
Tumor type				
Intestinal	49	23	46.9	< 0.05
Diffuse	35	9	25.7	

The proportion of patients with human epidermal growth factor receptor 2 (HER2) over-expression was higher in patients with gastric cancer (GC) of the intestinal-type than in those with GC of the diffuse-type.

Table 2 Clinicopathologic parameters and survival

Parameter	Patient (*n*)	2-yr survival (%)	*P* value
Age (yr)			> 0.05
< 60	56	66.1	
⩾ 60	28	60.7	
Gender			< 0.05
Male	66	78.6	
Female	18	21.4	
HER2 over-expression			< 0.05
Absent	49	58.3	
Present	35	41.7	
Lauren classification			< 0.05
Intestinal	49	58.3	
Diffuse	35	41.7	
Differentiation			< 0.05
High/middle	4	80	
Low/null	50	63.3	
Disease-free interval (n = 84)			< 0.05
⩾ 12 mo	63	75	
< 12 mo	19	22.6	

Disease-free survival longer than 12 mo has significant association with extended overall survival in gastric cancer patients with liver metastasis. HER2: Human epidermal growth factor receptor 2.

Statistical analysis

A total of 35 GC patients with HER2-positive liver metastases were included in this study. Liver metastatic GC was pathologically diagnosed by pathologists at the National Comprehensive Cancer Network. Patients with clinical symptoms suggesting liver metastasis were diagnosed by abdominal magnetic resonance imaging (MRI) or computed tomography (CT) clinical information was collected from medical records, including the date of initial diagnosis of GC, the development of liver metastases, the date of chemotherapy, and death or the last follow-up, as well as disease sites at the start of chemotherapy and the details of treatment. We also evaluated the clinical response of other metastatic diseases according to the Response Evaluation Criteria in Solid Tumors at the time of diagnosis of liver metastasis. The baseline characteristics and prognostic factors for GC were reviewed, including age, sex, differentiation and GC type, and disease-free interval.

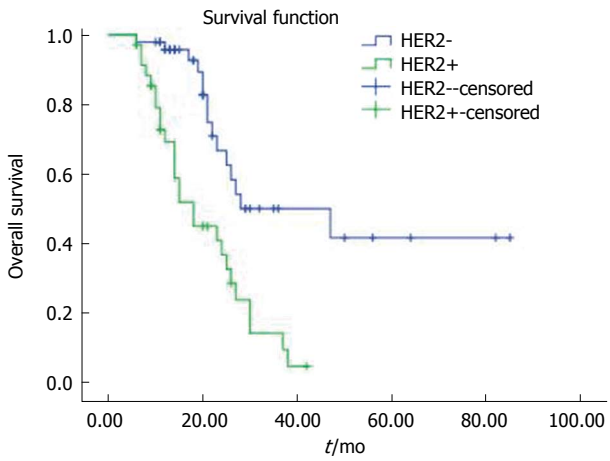

Figure 1 Kaplan-Meier survival curve in patients positive and negative for human epidermal growth factor receptor 2 in the liver metastatic gastric cancer. HER2: Human epidermal growth factor receptor 2.

Overall survival (OS) served as a main outcome.

SPSS version 17.0 software package (SPSS, Chicago, IL, United States) was used for statistical analysis. Frequency comparisons of HER2 expression status and clinicopathologic variables were performed with the F test (two-sided). Log-rank test was used for survival analysis. Survival curves were computed according to the Kaplan-Meier method. A value of $P < 0.05$ was considered statistically significant.

RESULTS

HER2 expression in the liver metastatic in gastric cancer patients

The median follow-up period was 47 mo (range: 6-85 mo). The characteristics of 35 (25.7%) patients with HER2 over-expression in liver metastatic GC are presented in Table 1. The results showed no significant differences between the sexes, although significant associations were observed between intestinal and diffuse ($P < 0.05$) types of GC. Over-expression of HER2 was observed as being higher for patients younger than 60 ($P < 0.05$).

HER2 over-expression was detected in 23 out of 49 (46.9%) patients with intestinal GC and 9 out of 35 (25.7%) patients with diffuse GC. The proportion of patients with HER2 over-expression was higher in patients with GC of the intestinal-type than in those with GC of the diffuse-type ($P < 0.05$). In addition, 29 out of 59 (49.2%) patients aged < 60 years were HER2-positive, while 8 out of 25 (32%) patients aged ⩾ 60 were HER2-positive, which was a significant difference ($P < 0.05$). However, there was no significant difference with regards to gender for HER2 over-expression (Table 1).

HER2 over-expression and overall survival

Univariate analysis (log-rank test) showed that HER2 over-expression, sex, Lauren classification, differentiation and disease-free interval were correlated with poor sur-

vival ($P < 0.05$), while age was not correlated with 2-year survival ($P > 0.05$) (Table 2). Survival analysis with a survival curve (Figure 1) showed that HER2 over-expression was significantly relevant with decreased survival time in GC patients with liver metastases ($P < 0.01$).

2-year survival was not associated with a patient's age. A disease-free survival (DFS) longer than 12 mo had significant association with an extended OS in GC patients with liver metastasis (Table 2).

The median survival time after diagnosis of liver metastases was 18 mo [95% confidence interval (CI): 9.07-26.94] among those HER2 positive with liver metastases in GC. In comparison, for 49 (69.4%) out of 84 HER2 negative patients with liver metastatic GC, the median survival time was 47 mo (95% CI: 19.37-74.63).

In patients with HER2 positive liver metastatic GC, the median OS was significantly shorter than in HER2 negative patients (median, 20.32 mo; 95% CI: 16.51-24.13 mo *vs* median, 50.14 mo; 95% CI: 37.83-62.45 mo; $P < 0.01$).

DISCUSSION

The HER2, an important member of the HER family, is encoded by a gene located on chromosome 17q21. During tumorigenesis, HER2 has been identified to act as an oncogene to modulate the proliferation, invasion and apoptosis of tumor cells. In cancer formation, HER2 acts as an oncogene to regulate the proliferation, invasion, and apoptosis of cancer cells. HER2 over-expression was reported in 9%-38% of GC, and was mainly found on the cell membrane[16]. The concordance between protein and mRNA over-expression of HER2 was recently elucidated in tumorigenesis, especially in cancers scored 3+ by IHC[17].

In approximately 25.7% of invasive GC, the HER2 tyrosine kinase receptor is over-expressed. HER2 consists of four different receptors and is associated with cell proliferation, differentiation, and survival. The HER2 over-expressing GC is more aggressive and has a poor prognosis. With respect to the importance of HER2 heterodimer in tumorigenesis, the signaling pathways and downstream effectors of the HER family have become key molecules in exploring strategies for anti-cancer therapy. For example, herceptin, a humanized monoclonal antibody targeting HER2/neu, has been used as a first-line anti-cancer drug in the treatment of breast cancer over-expressing HER2/neu[18,19]. Evidence from preclinical trials also indicated that herceptin could benefit GC patients[20,21].

To date, a high incidence of liver metastases has been observed in patients with HER2 over-expressing GC, which may be attributed to the biological characteristics of GC and the treatments for GC. A retrospective analysis has identified HER2 as a risk factor for the development of liver metastases and relapse. The 5-year incidence of liver metastases is significantly higher in patients with HER2 over-expressing GC. In particular, improvements in the treatment of systemic diseases have enabled patients with HER2 over-expressing metastatic GC to survive for a relatively long period of time. MRI and CT have been routinely used for the diagnosis of liver metastases in these patients and found that the incidence of liver metastases is increasing. However, the association between liver metastases and HER2 expression remains largely unclear.

The results of the present study demonstrated that 25.7% of GC patients with liver metastases had HER2 over-expression, and that HER2 was mostly expressed in the membrane, which is consistent with what has been previously reported. The present study aimed to investigate the predictive factors in GC patients with HER2 over-expressing liver metastases.

GC of the two histologic types (intestinal and diffuse) differs in their epidemiology, pathogenesis, clinical outcome, and even genetic profiling[22]. The study showed tumors of two Puerto Rican (PR) patients with overexpressed Her2/neu and the resulting partial clinical responses motivated us to compare Her2/neu expression in PR ($n = 101$) and Caucasian non-Hispanic ($n = 95$) patients. The immunohistochemistry of tumors showed overexpression of *P*-Stat3, Cyclin D1, and Her2/neu, compared to non-neoplastic mucosa. Her2/neu and EGF-R protein levels were statistically significantly different, with higher levels of both proteins in the PR group. Importantly, Her2/neu expression was strong and diffuse in tumors with signet-ring morphology, while other histo-pathological subtypes showed higher intra-tumoral Her2/neu heterogeneity than typically observed in breast cancer. Targeted therapies in gastric cancer directed at EGF-R and Hers-2/neu pathways warrant further investigation. These therapies may be especially effective in PR patients and in patients with signet-ring cell morphologies with a dismal prognosis[23]. The study showed that overexpression of HIF-1a had no association with clinicopathological status, patient prognosis, or chemosensitivity; the expression of HIF-1a mRNA is up-regulated by a signal transduction pathway from a tyrosine kinase receptor, such as HER2[24]. Our results showed HER2 positivity differed significantly by histologic subtype (intestinal: 46.9%; diffuse: 25.7%). The mechanisms of HER2 over-expression in intestinal-type GC are complex and still largely unclear. Depending on the depth of invasion (T), the involvement of lymph nodes (N), and the presence of distant metastasis (M), the TNM stage is the most important prognostic factor for GC in clinic practice[25]. The role of HER2 over-expression as a prognostic factor in GC is still controversial[26,27]. Recently, increasing evidence has showed a direct correlation between HER2 over-expression and poor survival[28]. In a series of 260 GC patients, Okegawa *et al*[29] found that HER2 over-expression was an independent factor and correlated with serosal invasion and lymph node metastases. Our results demonstrated that HER2 over-expression was closely associated with a lower survival rate in GC patients with liver metastases ($P < 0.01$). HER2 may become a novel molecule in tumorigenesis of GC and a potential candi-

date for molecule-targeted therapy, especially in diffuse-type GC.

In our study, liver metastases in GC patients with HER2-overexpression had a shorter DFS (75% *vs* 22.6%, $P < 0.05$), which was consistent with what had previously been reported[30]. Tumor type was significantly associated with liver metastases (intestinal 46.9% *vs* diffuse 25.7%, $P < 0.05$), but age was not associated with GC. The association between liver and other location metastases suggests that high tumor load contributes to the development of hematogenous metastases, and supports the results in the present study that GC in half of patients was refractory to systemic chemotherapy.

Patients with liver metastases had a shorter survival time than those without, and the median survival time of 18 mo was comparable with previously reported results. In our study, HER2 negative GC patients with liver metastases remained stable, in contrast to other location metastases at the time of diagnosis of liver metastases.

Furthermore, HER2 positive patients with liver metastases exhibited progressive bone and other metastases and 75% of them had a general condition that allowed further systemic therapy, from which most of them benefited. Therefore, although liver metastases seem to appear at the late stage in patients with GC, other metastatic foci are still more or less chemosensitive, and thus treating liver metastases has an important influence on prognosis.

In our study, HER2 over-expressing GC patients seemed to develop symptomatic liver metastases at about 2.5 years after the diagnosis of recurrent or metastatic GC, which poses the question as to whether liver metastases should be monitored. If surveillance is carried out, who would be the candidates and what timing for surveillance should be further investigated? To date, there has been no evidence to suggest that the surveillance of liver metastases in GC patients is beneficial for patient survival or cost-effectiveness.

In conclusion, our findings reveal that the liver metastases in GC patients with HER2-overexpression had a poor prognosis. The low survival was correlated with sex, HER2 over-expression, Lauren classification, differentiation, and disease-free interval. In our future studies, the optimal regimens for chemotherapy will be studied in GC patients with liver metastases who acquire prolonged OS.

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COMMENTS

Background

Gastric cancer (GC) is the second leading cause of cancer death worldwide, especially in Asia. There are many risk factors used for predicting the prognosis of GC patients. A high incidence of liver metastases has been reported among patients with human epidermal growth factor receptor 2 (HER2) over-expressed GC. However, there is some controversy regarding the hypothesis that HER2 expression serves as a factor for predicting the prognosis of GC patients.

Research frontiers

HER2, an important member of the HER family, is encoded by a gene located on chromosome 17q21. In cancer formation, HER2 acts as an oncogene to regulate the proliferation, invasion, and apoptosis of cancer cells. The current research hotspot is to study the risk factors for liver metastasis and the prognosis in patients with HER2 over-expressing GC.

Innovations and breakthroughs

Although HER2 is over-expressed about 38% of gastric cancer patients, few studies are available on HER2 status in the liver metastases of gastric carcinoma patients and there is also little data on the evaluated prognosis for Chinese patients. A total of 84 GC patients recruited from the General Hospital of the People's Liberation Army in China between 2003 and 2010 were randomly enrolled in this study. All patients underwent radical dissection and chemotherapy, and/or radiotherapy. HER2 over-expression was detected with immunohistochemistry in gastric cancer with liver metastasis patients. Liver metastasis was diagnosed by magnetic resonance imaging or computed tomography. The authors also evaluated the clinical response of other metastatic diseases according to the Response Evaluation Criteria in Solid Tumors at the time of diagnosis of liver metastasis. The baseline characteristics and prognostic factors for GC were reviewed, including age, sex, differentiation and GC type, and disease-free interval. Overall survival (OS) served as a main outcome. From the results, the authors drew the conclusion that HER2 over-expressing GC patients with liver metastases have a poor prognosis, and the overall survival was significantly lower in HER2 positive patients.

Applications

Immunohistochemistry may be used to screen the HER2 status in gastric carcinoma patients with liver metastasis patients, and to study the risk factors for liver metastasis in gastric cancer. Since the sample size of our study was not large enough, that limited this study's ability to provide robust evidence. Therefore, larger-scale, multicentric studies are needed to test the results. Even so, the authors believe the current study provides preliminary and powerful data of evaluating the prognosis in patients with HER2 over-expressing GC, and contributes to determining whether target therapy is necessary for gastric carcinoma with liver metastasis.

Terminology

HER2 is the human epidermal growth factor receptor 2, an important member of the HER family, that is encoded by a gene located on chromosome 17q21. During tumorigenesis, HER2 has been identified to act as an oncogene to modulate the proliferation, invasion and apoptosis of tumor cells.

Peer review

The authors provide a retrospective study which reviewed 84 patients with HER2 expressing liver metastases in gastric cancer between 2003 and 2010. The study shows that HER2-overexpression has no correlation with liver metastases in gastric cancer. However, OS was obviously lower for HER2 negative patients. HER2-overexpression had correlation to a lower survival rate. Overall, this is an interesting and good study.

REFERENCES

1 **Bulanov D**. [Gastric cancer - current state of the problem. Part I. Epidemiology. Pathology. Classification. Staging]. *Khirurgiia* (*Sofiia*) 2007; 48-59

2 **Yan SY**, Hu Y, Fan JG, Tao GQ, Lu YM, Cai X, Yu BH, Du YQ. Clinicopathologic significance of HER2/neu protein expression and gene amplification in gastric carcinoma. *World J Gastroenterol* 2011; **17**: 1501-1506

3 **Zhang XL**, Yang YS, Xu DP, Qu JH, Guo MZ, Gong Y, Huang J. Comparative study on overexpression of HER2/neu and HER3 in gastric cancer. *World J Surg* 2009; **33**: 2112-2118

4 **Yu GZ**, Chen Y, Wang JJ. Overexpression of Grb2/HER2 signaling in Chinese gastric cancer: their relationship with clinicopathological parameters and prognostic significance. *J Cancer Res Clin Oncol* 2009; **135**: 1331-1339

5 **Matsubara J**, Yamada Y, Nakajima TE, Kato K, Hamaguchi

T, Shirao K, Shimada Y, Shimoda T. Clinical significance of insulin-like growth factor type 1 receptor and epidermal growth factor receptor in patients with advanced gastric cancer. *Oncology* 2008; **74**: 76-83

6 **Tsugawa K**, Yonemura Y, Hirono Y, Fushida S, Kaji M, Miwa K, Miyazaki I, Yamamoto H. Amplification of the c-met, c-erbB-2 and epidermal growth factor receptor gene in human gastric cancers: correlation to clinical features. *Oncology* 1998; **55**: 475-481

7 **Brien TP**, Depowski PL, Sheehan CE, Ross JS, McKenna BJ. Prognostic factors in gastric cancer. *Mod Pathol* 1998; **11**: 870-877

8 **Gürel S**, Dolar E, Yerci O, Samli B, Oztürk H, Nak SG, Gülten M, Memik F. The relationship between c-erbB-2 oncogene expression and clinicopathological factors in gastric cancer. *J Int Med Res* 1999; **27**: 74-78

9 **Nakajima M**, Sawada H, Yamada Y, Watanabe A, Tatsumi M, Yamashita J, Matsuda M, Sakaguchi T, Hirao T, Nakano H. The prognostic significance of amplification and overexpression of c-met and c-erb B-2 in human gastric carcinomas. *Cancer* 1999; **85**: 1894-1902

10 **Allgayer H**, Babic R, Gruetzner KU, Tarabichi A, Schildberg FW, Heiss MM. c-erbB-2 is of independent prognostic relevance in gastric cancer and is associated with the expression of tumor-associated protease systems. *J Clin Oncol* 2000; **18**: 2201-2209

11 **García I**, Vizoso F, Martín A, Sanz L, Abdel-Lah O, Raigoso P, García-Muñiz JL. Clinical significance of the epidermal growth factor receptor and HER2 receptor in resectable gastric cancer. *Ann Surg Oncol* 2003; **10**: 234-241

12 **Jauch KW**, Heiss MM, Gruetzner U, Funke I, Pantel K, Babic R, Eissner HJ, Riethmueller G, Schildberg FW. Prognostic significance of bone marrow micrometastases in patients with gastric cancer. *J Clin Oncol* 1996; **14**: 1810-1817

13 **Maehara Y**, Yamamoto M, Oda S, Baba H, Kusumoto T, Ohno S, Ichiyoshi Y, Sugimachi K. Cytokeratin-positive cells in bone marrow for identifying distant micrometastasis of gastric cancer. *Br J Cancer* 1996; **73**: 83-87

14 **Matsunami K**, Nakamura T, Oguma H, Kitamura Y, Takasaki K. Detection of bone marrow micrometastasis in gastric cancer patients by immunomagnetic separation. *Ann Surg Oncol* 2003; **10**: 171-175

15 **Schlimok G**, Funke I, Pantel K, Strobel F, Lindemann F, Witte J, Riethmüller G. Micrometastatic tumour cells in bone marrow of patients with gastric cancer: methodological aspects of detection and prognostic significance. *Eur J Cancer* 1991; **27**: 1461-1465

16 **Tokunaga A**, Onda M, Okuda T, Teramoto T, Fujita I, Mizutani T, Kiyama T, Yoshiyuki T, Nishi K, Matsukura N. Clinical significance of epidermal growth factor (EGF), EGF receptor, and c-erbB-2 in human gastric cancer. *Cancer* 1995; **75**: 1418-1425

17 **Yano T**, Doi T, Ohtsu A, Boku N, Hashizume K, Nakanishi M, Ochiai A. Comparison of HER2 gene amplification assessed by fluorescence in situ hybridization and HER2 protein expression assessed by immunohistochemistry in gastric cancer. *Oncol Rep* 2006; **15**: 65-71

18 **de Graeff P**, Crijns AP, Ten Hoor KA, Klip HG, Hollema H, Oien K, Bartlett JM, Wisman GB, de Bock GH, de Vries EG, de Jong S, van der Zee AG. The ErbB signalling pathway: protein expression and prognostic value in epithelial ovarian cancer. *Br J Cancer* 2008; **99**: 341-349

19 **Rusnak DW**, Lackey K, Affleck K, Wood ER, Alligood KJ, Rhodes N, Keith BR, Murray DM, Knight WB, Mullin RJ, Gilmer TM. The effects of the novel, reversible epidermal growth factor receptor/ErbB-2 tyrosine kinase inhibitor, GW2016, on the growth of human normal and tumor-derived cell lines in vitro and in vivo. *Mol Cancer Ther* 2001; **1**: 85-94

20 **Kim SY**, Kim HP, Kim YJ, Oh do Y, Im SA, Lee D, Jong HS, Kim TY, Bang YJ. Trastuzumab inhibits the growth of human gastric cancer cell lines with HER2 amplification synergistically with cisplatin. *Int J Oncol* 2008; **32**: 89-95

21 **Inui T**, Asakawa A, Morita Y, Mizuno S, Natori T, Kawaguchi A, Murakami M, Hishikawa Y, Inui A. HER2 overexpression and targeted treatment by trastuzumab in a very old patient with gastric cancer. *J Intern Med* 2006; **260**: 484-487

22 **Roder DM**. The epidemiology of gastric cancer. *Gastric Cancer* 2002; **5** Suppl 1: 5-11

23 **Cangiano J**, Centeno BA, Garrett CR, Cáceres W, de Jesús A, Lee JH, Pavía O, Jove R, Báez L, Sullivan DM, Muro-Cacho CA, Muñoz-Antonia T. Signal transduction proteins in tumors from Puerto Rican and Caucasian gastric adenocarcinoma patients: expression differences with potential for specific targeted therapies. *Dig Dis Sci* 2008; **53**: 2090-2100

24 **Urano N**, Fujiwara Y, Doki Y, Tsujie M, Yamamoto H, Miyata H, Takiguchi S, Yasuda T, Yano M, Monden M. Overexpression of hypoxia-inducible factor-1 alpha in gastric adenocarcinoma. *Gastric Cancer* 2006; **9**: 44-49

25 **Yamashita K**, Sakuramoto S, Kikuchi S, Katada N, Kobayashi N, Watanabe M. Validation of staging systems for gastric cancer. *Gastric Cancer* 2008; **11**: 111-118

26 **Tateishi M**, Toda T, Minamisono Y, Nagasaki S. Clinicopathological significance of c-erbB-2 protein expression in human gastric carcinoma. *J Surg Oncol* 1992; **49**: 209-212

27 **Sasano H**, Date F, Imatani A, Asaki S, Nagura H. Double immunostaining for c-erbB-2 and p53 in human stomach cancer cells. *Hum Pathol* 1993; **24**: 584-589

28 **Fuse N**. [Relation of HER2 status and prognosis in gastric cancer patients]. *Gan To Kagaku Ryoho* 2011; **38**: 1073-1078

29 **Okegawa T**, Kinjo M, Nutahara K, Higashihara E. Pretreatment serum level of HER2/nue as a prognostic factor in metastatic prostate cancer patients about to undergo endocrine therapy. *Int J Urol* 2006; **13**: 1197-1201

30 **Kumada T**, Arai Y, Itoh K, Takayasu Y, Nakamura K, Ariyoshi Y, Tajima K. Phase II study of combined administration of 5-fluorouracil, epirubicin and mitomycin-C by hepatic artery infusion in patients with liver metastases of gastric cancer. *Oncology* 1999; **57**: 216-223

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BRIEF ARTICLE

Glypican-3 expression and its relationship with recurrence of HCC after liver transplantation

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Abstract

AIM: To investigate the diagnostic value of glypican-3 (GPC3) and its relationship with hepatocellular carcinoma (HCC) recurrence after liver transplantation.

METHODS: HCC tissue samples (n = 31) obtained from patients who had undergone liver transplantation were analyzed. GPC3 mRNA and protein expression were analyzed by TaqMan real-time reverse transcription-polymerase chain reaction and immunohistochemistry. Correlation between the GPC3 expression and clinicopathological features was analyzed. The potential prognostic value of GPC3 was investigated by comparing recurrence-free survival between HCC patients with and without GPC3 expression.

RESULTS: Using a cutoff value of 3.5×10^{-2}, 20 of 31 cancerous tissues had expression values of $> 3.5 \times 10^{-2}$, whereas 3 of 31 adjacent non-neoplastic parenchyma and 0 of 20 control liver tissues had expression values of $> 3.5 \times 10^{-2}$ ($P < 0.001$). GPC3 protein was immunoexpressed in 68% of cancerous tissues, but not in adjacent non-neoplastic parenchyma and control liver tissues. Vascular invasion was significantly related to GPC3 expression ($P < 0.05$). Recurrence-free survival was significantly longer for patients without GPC3 mRNA overexpression ($> 3.5 \times 10^{-2}$) and those without vascular invasion ($P < 0.05$ for both).

CONCLUSION: GPC3 expression may serve as a valuable diagnostic marker for HCC. GPC3 mRNA overexpression may be an adverse indicator for HCC patients after liver transplantation.

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Key words: Liver transplantation; Hepatocellular carcinoma; Glypican-3; mRNA; Recurrence

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common tumors in the world. More than 600 000 deaths globally per year have been reported, with 82% of cases (and deaths) occurring in "developing" countries (with 55% in China)[1,2]. HCC is up to four-times more common in men than in women, and 60%-90% of these tumors develop in

a cirrhotic liver[3]. About 200 000 patients die from HCC every year in China.

Liver transplantation offers the potential to resect the entire tumor-bearing liver and eliminate the cirrhosis. Liver transplantation therefore holds great theoretical appeal in treating this disease. Furthermore, the liver is entirely removed at liver transplantation, so recurrence must derive from extrahepatic dissemination that occurred before or during transplantation, reflecting highly aggressive tumor biology. Given the current shortage of organs and the risk of aggressive recurrence, selection of candidates for liver transplantation is crucial and controversial[4-6]. Therefore, predicting outcomes for patients is a challenge for clinicians and researchers.

Glypican-3 (GPC3) is a heparan sulfate proteoglycan and locates on the cell surface by a mechanism involving the glycosylphosphatidylinositol anchor[7]. GPC3 has been discovered to be a potential serologic and immunohistochemical diagnostic marker for HCC in general[8-10] and to promote the growth of HCC cells through stimulation of the canonical Wnt signaling pathway[11]. Zhu *et al*[12] and Sung *et al*[13] reported that GPC3 mRNA was significantly elevated in most HCC compared with normal liver as well as in livers with focal nodular hyperplasia and liver cirrhosis. This result was later confirmed by Libbrecht *et al*[14] using reverse transcription-polymerase chain reaction (RT-PCR), who mainly studied small HCC. Despite this clinical interest in GPC3, until now, the relationship between GPC3 expression and post-transplant HCC recurrence has not been clarified.

In our previous study, GPC3 mRNA-expressing cells in peripheral blood had no clinical value in the diagnosis of HCC[15]. The present study is an extension of the study mentioned above. We designed the study to assess the diagnostic value of GPC3 mRNA and protein expression in HCC tissues and to investigate their relationship with HCC recurrence after orthotopic liver transplantation (OLT).

MATERIALS AND METHODS

Patients

HCC tissue samples (n = 31) were obtained from patients who underwent OLT at the Department of Transplantation Surgery, Tianjin First Central Hospital (Tianjin, China) during 2008. Histological types were assigned according to the classification set by the World Health Organization. The clinicopathological features of OLT patients with HCC are summarized in Table 1. All recipients had undergone successful OLT (livers were from cadaveric donors who had agreed to donate their organs in the event of death). Liver-tissue specimens were obtained intraoperatively from cancerous tissues and adjacent non-neoplastic parenchyma. The controls were normal liver tissues from 20 patients (15 men, 5 women; mean age, 48 years; age range 30-65 years) with hepatic hemangioma. There were no statistically significant differences in age and sex between the study groups ($P > 0.05$). Liver-tissue specimens were fixed in 10 % formalin and embedded in paraffin for routine histological examination. The remaining tissue was immediately snap-frozen in liquid nitrogen and stored at -80 ℃ until examination.

Table 1 Clinicopathological features of the study population (n = 31)

Gender (Male/female)	29/2
Age (mean/range) (yr)	49/37-62
Primary liver disease (HBV cirrhosis/HCV cirrhosis)	30/1
TNM stage (Ⅰ-Ⅱ/Ⅲ-Ⅳa)	12/19
Milan criteria (within/beyond)	15/16
Tumor diameter (< 5 cm/≥ 5 cm)	16/15
Tumor number (< 3/≥ 3)	26/5
Vascular invasion (present/absent)	16/15
Serum AFP (≤ 20 μg/L/> 20 μg/L)	11/20
Histological differentiation (good/moderate and poor)	6/25

HBV: Hepatitis B virus; HCV: Hepatitis C virus; TNM: Tumor, node and metastasis; AFP: α fetoprotein.

All patients received standard post-liver transplantation care in the Intensive Care Unit. They received the same immunosuppressive therapy: tacrolimus, daclizumab, mycophenolate mofetil, and methylprednisolone. The diagnosis of HCC recurrences (follow-up of 24 mo) was based on elevation of serum levels of alpha-fetoprotein (AFP) and ultrasonography, hepatic arteriography, or computed tomography. Fine-needle aspiration cytology was used for confirmation of recurrence if necessary.

The present study was approved by our institutional ethics committee. Informed consent was obtained from each patient. The procedure met all applicable guidelines of our institute as well as governmental regulations concerning the ethical use of donated organs.

Real time reverse transcription-polymerase chain reaction assay

Total RNA was extracted from frozen liver tissues using Trizol reagents according to manufacturer instructions (Invitrogen, Carlsbad, CA, United States). Reverse transcription to complementary DNA (cDNA) was undertaken using random primer and Superscript RNase H-reverse transcriptase (Invitrogen).

A total of 1 μL of cDNA was mixed with the TaqMan probe[15], RNase-free water, and TaqMan Universal PCR Master Mix. Real-time PCR amplification and data analyses were carried out using an ABI PRISM 7300 Sequence Detector System (Applied Biosystems, Foster City, CA, United States) according to the manufacturer's protocol. β-actin was used as an internal control. Each sample was assayed in duplicate in a MicroAmp optical 96-well plate. The thermo-cycling condition was 2 min at 50 ℃ and 15 min incubation at 95 ℃, followed by 40 two-temperature cycles of 20 s at 95 ℃ and 1 min at 60 ℃. Data were analyzed with Sequence Detection Software. Mean and standard-deviation values were calculated from the data obtained. The level of expression was

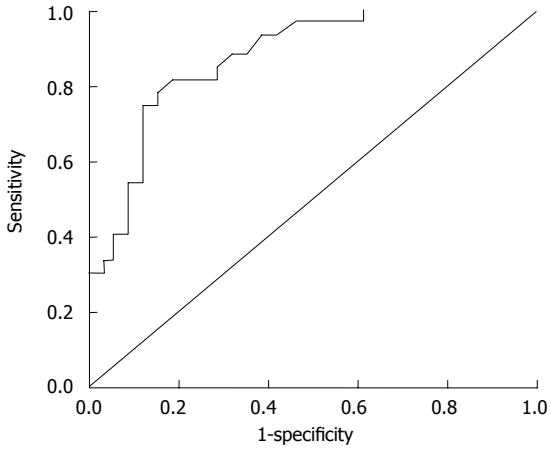

Figure 1 Receiver operating characteristic curve of glypican-3 mRNA levels for the detection of hepatocellular carcinoma. The area under the receiver operating characteristic curve is 0.878 (95% confidence interval: 0.802-0.953) ($P < 0.001$).

calculated using the formula: Relative expression = (copy number of target molecule/copy number of β-actin)[16].

Immunohistochemical analyses

Sections of formalin-fixed, paraffin-embedded tissue cell blocks (4 μm) were tested for the presence of monoclonal antibody directed against GPC3 (Santa Cruz Biotechnology, Santa Cruz, CA, United States). The method used for the immunohistochemical analysis has been described previously[17].

For the immunohistochemical analysis of GPC3, we evaluated the area of GPC3-positive staining in one slide for each patient. At first, to analyze GPC3 expression, the results of immunohistochemical staining were classified according to the area of GPC3-positive stained cells as follows: -, Negative (< 10%); ±, Weakly positive (10%-30%); and +, Positive (> 30%). Finally, we classified two groups between GPC3-negative (< 10%) and GPC3-positive (> 10%). The expression of GPC3 was judged to be positive if the percentage of immunoreactive cells was assessed semi-quantitatively as being ⩾ 10% in focal lesions.

Statistical analysis

Data are mean ± SD. For comparisons of continuous variables, one-way analysis of variance with the Bonferroni *post-hoc* test was used to compare differences among the three groups. We generated receiver operating characteristic curves for GPC3 mRNA levels to determine the cutoff points that yielded the sensitivity and specificity for predicting the diagnosis of HCC. Correlations between GPC3 results and various clinicopathological parameters in the tissue samples were evaluated using the chi-square test. The distribution of time to recurrence was estimated according to the Kaplan-Meier method, and differences assessed using log-rank statistics. $P < 0.05$ was considered significant. Statistical analyses were done using SPSS ver12 (SPSS, Chicago, IL, United States).

Table 2 Correlation between glypican-3 expression and clinicopathological features in 31 orthotopic liver transplantation patients with hepatocellular carcinoma

Variable	GPC3 protein expression (*n*/N) (%)	*P* value	GPC3 mRNA overexpression (*n*/N) (%)	*P* value
TNM stage				
Ⅰ-Ⅱ	7/12 (58)		6/12 (50)	
Ⅲ-Ⅳa	14/19 (74)	0.373	14/19 (74)	0.179
Milan criteria				
Within	11/15 (73)		8/15 (53)	
Beyond	10/16 (62)	0.519	12/16 (75)	0.208
Tumor diameter				
< 5 cm	11/16 (69)		9/16 (56)	
⩾ 5 cm	10/15 (67)	0.901	11/15 (73)	0.320
Tumor number				
< 3	18/26 (69)		17/26 (65)	
⩾ 3	3/5 (60)	0.686	3/5 (60)	0.818
Vascular invasion				
Present	14/16 (87)		14/16 (87)	
Absent	7/15 (47)	0.015	6/15 (40)	0.006
Serum AFP				
⩽ 20 μg/L	6/11 (54)		6/11 (54)	
> 20 μg/L	15/20 (75)	0.244	14/20 (70)	0.390
Histological differentiation				
Good	3/6 (50)		2/6 (33)	
Moderate and poor	18/25 (72)	0.301	18/25 (72)	0.075

TNM: Tumor, node and metastasis; AFP: α fetoprotein; GPC3: Glypican-3.

RESULTS

Glypican-3 mRNA expression in hepatocellular carcinoma tissues

GPC3 mRNA expression in adjacent non-neoplastic parenchyma ($n = 31$, $2.00 \pm 1.91 \times 10^{-2}$) and in control liver tissues ($n = 20$, $1.41 \pm 1.57 \times 10^{-2}$) was much lower than in cancerous tissues ($n = 31$, $5.64 \pm 3.06 \times 10^{-2}$) ($P < 0.001$). There were no significant differences between adjacent non-neoplastic parenchyma and normal liver tissues with respect to GPC3 mRNA expression ($P = 0.270$). A cutoff value for GPC3 mRNA expression of 3.5×10^{-2} was diagnostic of HCC with a sensitivity of 68%, specificity of 94%, positive predictive value (PPV) of 87%, and negative predictive value (NPV) of 83% [area under the curve, 0.878; 95% confidence interval (CI): 0.802-0.953, $P < 0.001$] (Figure 1). Twenty of 31 (64%) cancerous tissues had expression values of $> 3.5 \times 10^{-2}$, whereas 3 of 31 (10%) adjacent non-neoplastic parenchyma and 0 of 20 control liver tissues had expression values of $> 3.5 \times 10^{-2}$ ($P < 0.001$).

The expression of GPC3 mRNA was evaluated in relation to clinicopathological features. GPC3 mRNA overexpression ($> 3.5 \times 10^{-2}$) was closely associated with vascular invasion ($P = 0.006$). No significant correlation was found between GPC3 mRNA overexpression and tumor, node, metastasis (TNM) stages, Milan criteria, tumor diameter, tumor number, serum AFP levels, and histological differentiation (Table 2).

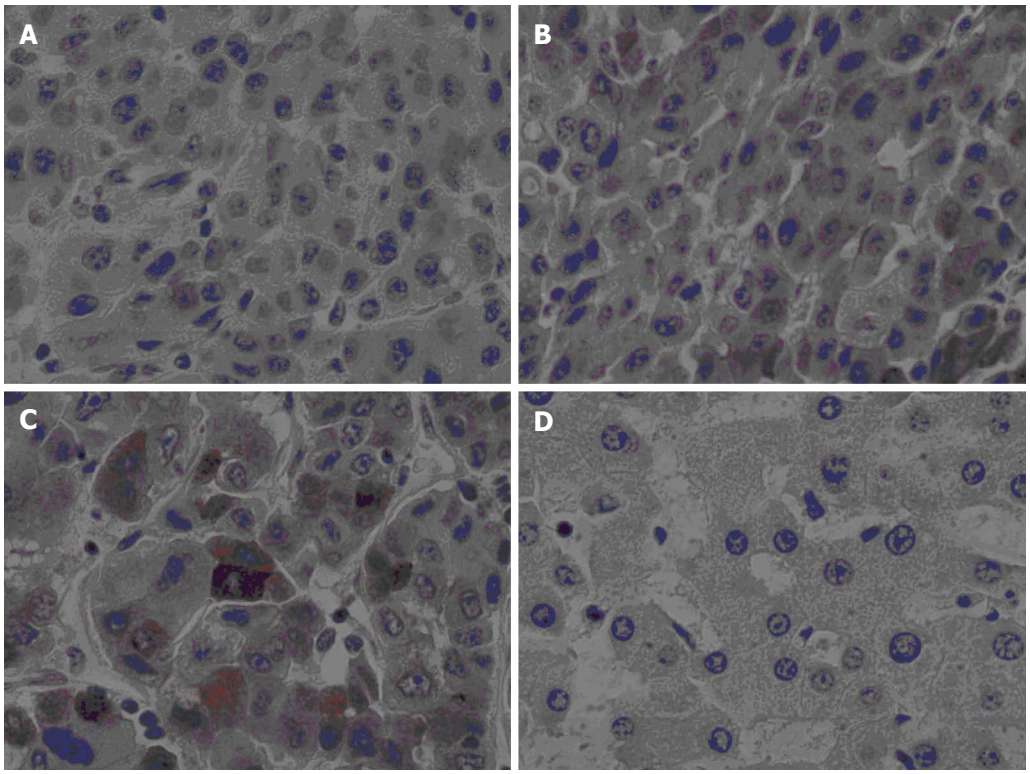

Figure 2 Hepatocellular carcinoma immunohistochemical staining (× 400). Glypican-3 expression in hepatocellular carcinoma by immunohistochemical analyses. A: Negative expression in HCC tissue; B: Weakly positive expression in HCC tissue; C: Positive expression in HCC tissue; D: Negative expression in adjacent non-neoplastic parenchyma. HCC: Hepatocellular carcinoma.

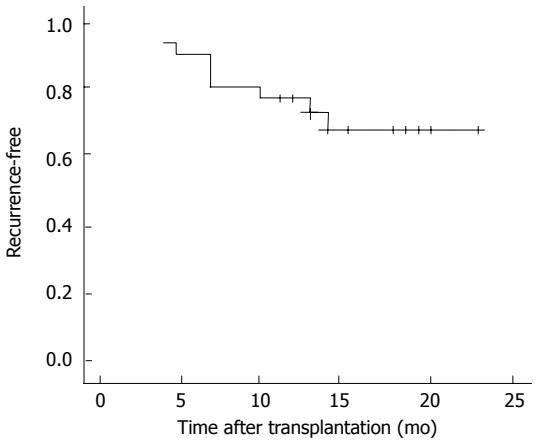

Figure 3 Kaplan-Meier survival curve for recurrence-free interval in 31 patients who underwent liver transplantation.

GPC3 protein expression in hepatocellular carcinoma tissues

GPC3 protein was immunoexpressed in 21 of 31 (68%) cancerous tissues, but not in any adjacent non-neoplastic parenchyma and control liver tissues ($P < 0.001$). In GPC3-positive cases, protein expression was localized in the cytoplasm and membrane of cells (Figure 2). The sensitivity, specificity, PPV, and NPV of GPC3 protein expression was 0.68, 1.0, 1.0, and 0.84. Vascular invasion was significantly related to GPC3 protein expression ($P = 0.015$) (Table 2).

Association between glypican-3 expression and hepatocellular carcinoma recurrence

Up-to-date analyses after a follow-up of 24 mo revealed that 10 (32%) patients developed HCC recurrence. The median duration of HCC recurrence was 8.1 (range, 3-18 mo) after OLT (Figure 3). Assessment of Kaplan-Meier curves showed that patients without GPC3 mRNA overexpression had significantly longer recurrence-free survival than those with GPC3 mRNA overexpression ($P = 0.034$, log-rank test) (Figure 4A). Recurrence-free survival was significantly longer in patients without evidence of vascular invasion compared with those with vascular invasion ($P = 0.016$, log-rank test). Patients who met the Milan criteria had longer recurrence-free survival than those who did not, though the difference was not statistically significant ($P = 0.077$, log-rank test). There was no significant difference in recurrence-free survival between patients with positive and negative GPC3 protein expression ($P = 0.231$, log-rank test) (Figure 4B).

DISCUSSION

OLT is an excellent therapeutic choice for patients with cirrhosis complicated by HCC because it provides simultaneous treatment for both diseases. However, due to the limited availability of organs, prior selection of candidates most likely to benefit from OLT is very important. Thus, screening for potential early prognostic markers and therapeutic targets is very urgent for better selection

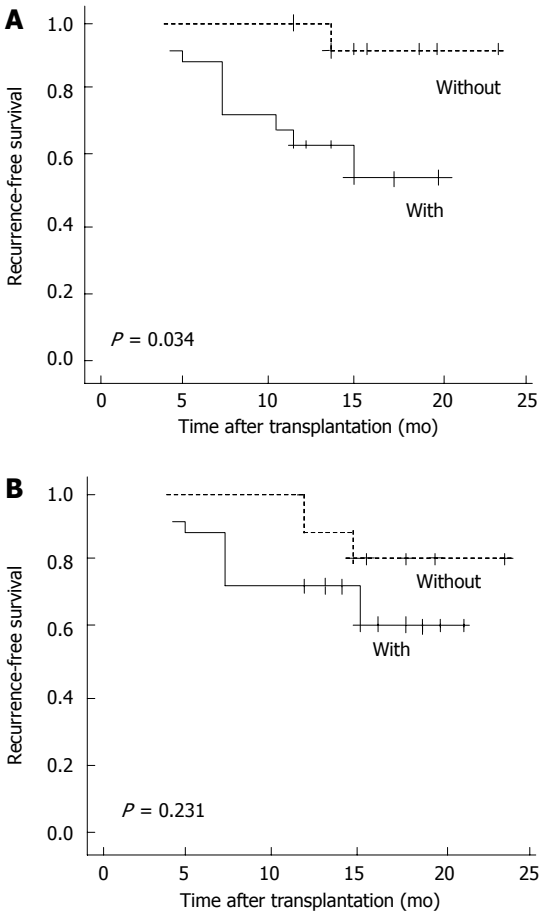

Figure 4 Kaplan-Meier survival curves for recurrence-free survival after liver transplantation for hepatocellular carcinoma. A: with or without glypican-3 mRNA overexpression (> 3.5 × 10^{-2}) (P = 0.034, compared by log-rank test); B: with or without glypican-3 protein expression (P = 0.231, compared by log-rank test).

of HCC patients for liver transplantation.

More recent studies have demonstrated upregulation of GPC3 in HCC at the protein level. GPC3 expression has been noted in 57% to 90% of HCC cases using immunohistochemistry[17-20]. In the present study, immunohistochemical analyses revealed that HCC expressed GPC3 protein in 68% of HCC tissues tested. RT-PCR analyses showed that, with a cutoff value of 3.5 × 10^{-2}, 20 of 31 HCC tumors showed much stronger expression of GPC3 mRNA than adjacent non-neoplastic parenchyma. It was proposed that GPC3 expression could be a useful tissue marker to distinguish between benign and malignant hepatocellular lesions. Nevertheless, in some studies, HCC and non-malignant liver originated from different patients, so these studies could not exclude the possibility that HCC and adjacent non-neoplastic parenchyma have similar GPC3 expression[12,21]. We can presume that retained carcinoma cells in the adjacent non-neoplastic parenchyma of surgical specimens with large tumors and tumor invasion may be the cause of GPC3 protein expression or GPC3 mRNA overexpression. This suggests that detection of GPC3 expression in adjacent non-neoplastic parenchyma could indicate whether or not the tumor is completely resected. It may also serve as a reference value in deciding if further treatment after resection is required.

Although GPC3 mRNA and protein show expression in HCC tissues, in our previous study, we examined GPC3 mRNA-expressing cells in peripheral blood in liver-transplant recipients during the transplant period, and found no clinical diagnostic value for HCC. Studies showed that GPC3 was a potential serologic diagnostic marker for HCC. However, the diagnostic value of GPC3 protein in sera remains questionable. Yasuda *et al*[22] found that GPC3 is not a serologic marker for detection of HCC. We speculate that GPC3 serves as a potential tissue-specific diagnostic marker for HCC. Some liver-transplant candidates undergo locoregional tumor treatments, so obtaining tissue samples for preoperative molecular marker analyses would not be problematic.

In the clinicopathological analysis, TNM stages, Milan criteria, tumor diameter, tumor number, or serum AFP levels were not clearly associated with increase in GPC3 expression. GPC3 may therefore play an important part in the malignant transformation of cells in HCC. Up until now, AFP has been regarded as the most useful marker of HCC, although its sensitivity is limited[23]. Our data show that GPC3 mRNA and protein expression in HCC without serum AFP elevation (≤ 20 μg/L) were 54% and 54%, respectively, and in HCC of diameter < 5 cm were 56% and 69%, respectively. These findings suggest that GPC3 is a sensitive marker in small HCC and negative-serum AFP patients not only in resection[24,25] but also in OLT. Vascular invasion has been consistently reported to be a significant factor for a poor prognosis after liver transplantation for HCC[15,26,27]. Interestingly, in the present study, vascular invasion was significantly related to GPC3 expression. Zhu *et al*[12] using *in-situ* hybridization signals, showed that pushing HCC expressed significantly more GPC3 mRNA than the invading HCC. Their observations suggested that GPC3 may promote the growth of local cancer cells, and may also inhibit tissue invasion and metastasis. Their finding is different to ours. In addition, expression of GPC3 was lower in well-differentiated HCC than in moderately and poorly differentiated HCC, though the difference was not statistically significant, a finding consistent with previous reports[17,19]. The present study suggests that GPC3 expression may facilitate the growth of cancer cells, the degree of malignancy, and contribution to HCC progression.

Recurrence of HCC after transplantation remains a formidable problem despite refined selection criteria and exhaustive preoperative staging. In the present study, recurrence-free survival was longer in patients who had tumors with no evidence of vascular invasion. Microscopic vascular invasion in the explant is known to be a risk factor for HCC recurrence after transplantation. In addition, recurrence-free survival was longer in patients who met the Milan criteria, though the difference was not statistically significant. This could be because (1) the sample size in the present study was relatively small to allow apprecia-

tion of significant differences in the frequency of HCC recurrence in the groups; and (2) the patients in the Milan group had worse pathological stages. In our previous study, GPC3 mRNA-expressing cells in peripheral blood carried no predictive value for HCC recurrence after liver transplantation[15]. Hence, we used liver tissue instead of peripheral blood to determine if GPC3 expression in tumor tissues was associated with HCC recurrence after liver transplantation. In the present study, HCC patients with GPC3 mRNA overexpression had a significantly shorter recurrence-free survival than those with lower GPC3 mRNA expression after liver transplantation. This finding indicated that GPC3 mRNA overexpression could be an independent factor of a poor prognosis in HCC. Thus, we hypothesized that GPC3 may have a role in promoting carcinogenesis and in the development of HCC.

In general, GPC3 has been reported to interfere with different pathways and growth factors, and has a tissue- and stage-specific role in the development and tumor growth[28]. Ishiguro *et al*[29] showed that anti-GPC3 antibody can be used as a potential antitumor agent for human liver cancer, and can provide a novel treatment option for liver-cancer patients with GPC3-positive tumors. Evaluation of GPC3 as a diagnostic and immunotherapeutic target may be worthwhile for the prevention and treatment of HCC. Further studies must be completed to clarify these issues.

In conclusion, GPC3 is a valuable diagnostic marker sensitive and specific for HCC in liver-transplant patients. The most valuable finding in the present study was that GPC3 mRNA overexpression may act as a prognostic factor for recurrence-free survival in HCC after liver transplantation. The number of samples used in the present study was small. Confirmation of the findings of our study using more samples is warranted.

COMMENTS

Background

Given the current shortage of organs and the risk of aggressive recurrence, selection of candidates for liver transplantation is crucial and controversial. Establishment of direct and accurate methods to detect hepatocellular carcinoma (HCC) and predict HCC recurrence after liver transplantation is needed.

Research frontiers

Glypican-3 (GPC3) has been discovered to be a potential serologic and immunohistochemical diagnostic marker for HCC. Despite clinical interest in GPC3, until now, the relationship between GPC3 expression and post-transplant HCC recurrence has not been clarified.

Innovations and breakthroughs

In the previous study, the level of GPC3 mRNA in peripheral blood had no clinical value in assessing tumor recurrence after liver transplantation. Therefore, the present study further examined the diagnostic value of GPC3 expression as retrospectively analyzed by real-time reverse transcription- polymerase chain reaction and immunohistochemistry in explant tumor samples. The principal finding was that GPC3 mRNA overexpression may act as an adverse indicator for HCC patients after liver transplantation because it was associated with significantly shorter recurrence-free survival.

Applications

The results of the present study suggest that GPC3 is a valuable diagnostic marker sensitive and specific for HCC in liver-transplant patients. Also, GPC3 mRNA overexpression may act as a prognostic factor for recurrence-free survival in HCC after liver transplantation.

Terminology

GPC3 is a heparan sulfate proteoglycan which is bound to the cell surface by a mechanism involving the glycosylphosphatidylinositol anchor. GPC3 is highly expressed in fetal but not in normal adult liver.

Peer review

Authors focused on the role of GPC3 mRNA and protein expression in the diagnosis of recurring HCC after liver transplantation. For this purpose, patient samples were analyzed according to clinicopathological features, mRNA expression levels, protein expression, and recurrence-free survival. They concluded that GPC3 expression could serve as a diagnostic marker for HCC and as an adverse indicator for HCC patients after liver transplantation.

REFERENCES

1 **Gomaa AI**, Khan SA, Toledano MB, Waked I, Taylor-Robinson SD. Hepatocellular carcinoma: epidemiology, risk factors and pathogenesis. *World J Gastroenterol* 2008; **14**: 4300-4308

2 **El-Serag HB**, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; **132**: 2557-2576

3 **Stefaniuk P**, Cianciara J, Wiercinska-Drapalo A. Present and future possibilities for early diagnosis of hepatocellular carcinoma. *World J Gastroenterol* 2010; **16**: 418-424

4 **Zavaglia C**, De Carlis L, Alberti AB, Minola E, Belli LS, Slim AO, Airoldi A, Giacomoni A, Rondinara G, Tinelli C, Forti D, Pinzello G. Predictors of long-term survival after liver transplantation for hepatocellular carcinoma. *Am J Gastroenterol* 2005; **100**: 2708-2716

5 **Silva MF**, Wigg AJ. Current controversies surrounding liver transplantation for hepatocellular carcinoma. *J Gastroenterol Hepatol* 2010; **25**: 1217-1226

6 **Schwartz ME**, D'Amico F, Vitale A, Emre S, Cillo U. Liver transplantation for hepatocellular carcinoma: Are the Milan criteria still valid? *Eur J Surg Oncol* 2008; **34**: 256-262

7 **Song HH**, Filmus J. The role of glypicans in mammalian development. *Biochim Biophys Acta* 2002; **1573**: 241-246

8 **Capurro M**, Wanless IR, Sherman M, Deboer G, Shi W, Miyoshi E, Filmus J. Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. *Gastroenterology* 2003; **125**: 89-97

9 **Kandil DH**, Cooper K. Glypican-3: a novel diagnostic marker for hepatocellular carcinoma and more. *Adv Anat Pathol* 2009; **16**: 125-129

10 **Tangkijvanich P**, Chanmee T, Komtong S, Mahachai V, Wisedopas N, Pothacharoen P, Kongtawelert P. Diagnostic role of serum glypican-3 in differentiating hepatocellular carcinoma from non-malignant chronic liver disease and other liver cancers. *J Gastroenterol Hepatol* 2010; **25**: 129-137

11 **Capurro MI**, Xiang YY, Lobe C, Filmus J. Glypican-3 promotes the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling. *Cancer Res* 2005; **65**: 6245-6254

12 **Zhu ZW**, Friess H, Wang L, Abou-Shady M, Zimmermann A, Lander AD, Korc M, Kleeff J, Büchler MW. Enhanced glypican-3 expression differentiates the majority of hepatocellular carcinomas from benign hepatic disorders. *Gut* 2001; **48**: 558-564

13 **Sung YK**, Hwang SY, Park MK, Farooq M, Han IS, Bae HI, Kim JC, Kim M. Glypican-3 is overexpressed in human hepatocellular carcinoma. *Cancer Sci* 2003; **94**: 259-262

14 **Libbrecht L**, Severi T, Cassiman D, Vander Borght S, Pirenne J, Nevens F, Verslype C, van Pelt J, Roskams T. Glypican-3 expression distinguishes small hepatocellular carcinomas from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like nodules. *Am J Surg Pathol* 2006; **30**: 1405-1411

15 **Wang Y**, Shen Z, Zhu Z, Han R, Huai M. Clinical values of AFP, GPC3 mRNA in peripheral blood for prediction of hepatocellular carcinoma recurrence following OLT: AFP, GPC3 mRNA for prediction of HCC. *Hepat Mon* 2011; **11**: 195-199

16 **Wang YL**, Li G, Wu D, Liu YW, Yao Z. Analysis of alpha-fetoprotein mRNA level on the tumor cell hematogenous spread of patients with hepatocellular carcinoma undergoing orthotopic liver transplantation. *Transplant Proc* 2007; **39**: 166-168
17 **Shirakawa H**, Suzuki H, Shimomura M, Kojima M, Gotohda N, Takahashi S, Nakagohri T, Konishi M, Kobayashi N, Kinoshita T, Nakatsura T. Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma. *Cancer Sci* 2009; **100**: 1403-1407
18 **Nassar A**, Cohen C, Siddiqui MT. Utility of glypican-3 and survivin in differentiating hepatocellular carcinoma from benign and preneoplastic hepatic lesions and metastatic carcinomas in liver fine-needle aspiration biopsies. *Diagn Cytopathol* 2009; **37**: 629-635
19 **Shirakawa H**, Kuronuma T, Nishimura Y, Hasebe T, Nakano M, Gotohda N, Takahashi S, Nakagohri T, Konishi M, Kobayashi N, Kinoshita T, Nakatsura T. Glypican-3 is a useful diagnostic marker for a component of hepatocellular carcinoma in human liver cancer. *Int J Oncol* 2009; **34**: 649-656
20 **Liu H**, Li P, Zhai Y, Qu CF, Zhang LJ, Tan YF, Li N, Ding HG. Diagnostic value of glypican-3 in serum and liver for primary hepatocellular carcinoma. *World J Gastroenterol* 2010; **16**: 4410-4415
21 **Midorikawa Y**, Ishikawa S, Iwanari H, Imamura T, Sakamoto H, Miyazono K, Kodama T, Makuuchi M, Aburatani H. Glypican-3, overexpressed in hepatocellular carcinoma, modulates FGF2 and BMP-7 signaling. *Int J Cancer* 2003; **103**: 455-465
22 **Yasuda E**, Kumada T, Toyoda H, Kaneoka Y, Maeda A, Okuda S, Yoshimi N, Kozawa O. Evaluation for clinical utility of GPC3, measured by a commercially available ELISA kit with Glypican-3 (GPC3) antibody, as a serological and histological marker for hepatocellular carcinoma. *Hepatol Res* 2010; **40**: 477-485
23 **Farinati F**, Marino D, De Giorgio M, Baldan A, Cantarini M, Cursaro C, Rapaccini G, Del Poggio P, Di Nolfo MA, Benvegnù L, Zoli M, Borzio F, Bernardi M, Trevisani F. Diagnostic and prognostic role of alpha-fetoprotein in hepatocellular carcinoma: both or neither? *Am J Gastroenterol* 2006; **101**: 524-532
24 **Yu MC,** Lee YS, Lin SE, Wu HY, Chen TC, Lee WC, Chen MF, Tsai CN. Recurrence and Poor Prognosis Following Resection of Small Hepatitis B-Related Hepatocellular Carcinoma Lesions Are Associated with Aberrant Tumor Expression Profiles of Glypican 3 and Osteopontin. *Ann Surg Oncol* 2011; Epub ahead of print
25 **Shafizadeh N**, Ferrell LD, Kakar S. Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the differentiation spectrum. *Mod Pathol* 2008; **21**: 1011-1018
26 **Sauer P**, Kraus TW, Schemmer P, Mehrabi A, Stremmel W, Buechler MW, Encke J. Liver transplantation for hepatocellular carcinoma: is there evidence for expanding the selection criteria? *Transplantation* 2005; **80**: S105-S108
27 **Cheung ST**, Fan ST, Lee YT, Chow JP, Ng IO, Fong DY, Lo CM. Albumin mRNA in plasma predicts post-transplant recurrence of patients with hepatocellular carcinoma. *Transplantation* 2008; **85**: 81-87
28 **Filmus J**, Capurro M. The role of glypican-3 in the regulation of body size and cancer. *Cell Cycle* 2008; **7**: 2787-2790
29 **Ishiguro T**, Sugimoto M, Kinoshita Y, Miyazaki Y, Nakano K, Tsunoda H, Sugo I, Ohizumi I, Aburatani H, Hamakubo T, Kodama T, Tsuchiya M, Yamada-Okabe H. Anti-glypican 3 antibody as a potential antitumor agent for human liver cancer. *Cancer Res* 2008; **68**: 9832-9838

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BRIEF ARTICLE

Salvage liver transplantation in the treatment of hepatocellular carcinoma: A Meta-analysis

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Abstract

AIM: To evaluate survival and recurrence after salvage liver transplantation (SLT) for the treatment of hepatocellular carcinoma (HCC) compared with primary liver transplantation (PLT) using a meta-analysis.

METHODS: Literature on SLT versus PLT for the treatment of HCC published between 1966 and July 2011 was retrieved. A meta-analysis was conducted to estimate pooled survival and disease-free rates. A fixed or random-effect model was established to collect the data.

RESULTS: The differences in overall survival and disease-free survival rates at 1-year, 3-year and 5-year survival rates were not statistically significant between SLT group and PLT group ($P > 0.05$). After stratifying the various studies by donor source and Milan criteria, we found that: (1) Living donor liver transplantation recipients had significantly higher 1-year survival rate, lower 3-year and 5-year survival rates compared with deceased-donor liver transplantation (DDLT) recipients. And in DDLT recipients they had better 1-year and 5-year disease-free survival rate in SLT group; and (2) No difference was seen in 1-year, 3-year and 5-year survival rates between two groups who beyond Milan criteria at the time of liver transplantation.

CONCLUSION: SLT can be effectively performed for patients with recurrence or deterioration of liver function after hepatectomy for HCC. It does not increase the perioperative mortality and has a similar long-term survival rates compared to PLT.

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Key words: Salvage liver transplantation; Primary liver transplantation; Hepatocellular carcinoma; Meta-analysis; Survival rate

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INTRODUCTION

In a normal liver, liver resection for hepatocellular carcinoma (HCC) is the primary treatment of choice. But in cirrhotic livers, the presence of HCC and the limited liver capacity are the two intertwined issues rendering the HCC unresectable. Primary liver transplantation (PLT) is the most effective treatment for such HCC patients, especially for those who meet Milan criteria (solitary liver nodule not exceeding 5 cm in maximum diameter, or 2 or 3 tumors not exceeding 3 cm in diameter)[1]. It has been manifested to provide a considerable disease-free

survival and to be the first choice for these patients. Due to shortage of available donors, long waiting times may harm the benefit that might be acquired from PLT. Salvage liver transplantation (SLT) has been proposed and performed for those who undergo primary liver resection for HCC or HCC recurrence or deterioration of liver function[2]. SLT proposes liver resection as a bridge to prevent tumor progression in the waiting list. Although SLT might be an alternate choice for HCC patients as the preferred treatment, long-term results are difficult to ascertain. Moreover, few data are available on the overall and disease-free survival of patients. A few researches concern on the comparison between the result of SLT and PLT. In order to reduce research bias and difference, we did a meta-analysis to compare survival and recurrences for SLT strategy versus PLT in the treatment of HCC patients, in order to provide a reference for clinical practice.

MATERIALS AND METHODS

Literature search strategy

Search was applied to the following electronic databases: PubMed (1966 to July 2011), Embase (January 1996 to July 2011), CNKI (January 1996 to July 2011) and Cochrane database. The following key words were used: "liver resection" or "hepatectomy"; "liver transplantation" or "transplantation" or "salvage liver transplantation" or "salvage transplantation"; "hepatocellular carcinoma" or "HCC". The search was limited to the English language and humans. The relevant reference lists of reviews were also searched at the same time. Abstracts or unpublished studies were not considered. If more than 1 study was published by the same author using the same case series, only the most detailed study was included. And if necessary, authors were contacted to obtain more data on their study.

Definitions

SLT was defined as a liver transplantation performed for recurrent HCC or deterioration of liver function after primary liver resection.

Inclusion, exclusion criteria and quality of the studies

Inclusion criteria were as follows: (1) having definition of SLT; (2) follow-up 12 mo at least; (3) case-control or cohort design; and (4) sufficient data were obtained to calculate odds ratio (OR) with confidence interval (CI). Reasons for exclusion were: (1) no-control; (2) duplicate; and (3) no useable data reported. We also excluded articles published before 1996 because there was no definition for "SLT".

The scoring system was adapted from Stahl, the Cochrane Collaboration and others[3-5]. This system suits not only randomized control trial (RCT) but controlled trial or other studies well. Questions were placed on a 3 point scale: unclear/inadequate (0), adequate (1), good (2). Articles were considered for inclusion if their summary score exceeded 30.

Table 1 Details of studies included in the meta-analysis

Author	Year	Country	Study design	SLT (cases)	PLT (cases)	Score
Adam *et al*[6]	2003	France	Case-control	17	200	32
Belghiti *et al*[7]	2003	France	Case-control	18	70	33
Concejero *et al*[8]	2008	China	Case-control	7	28	31
Del Gaudio *et al*[9]	2008	Italy	Case-control	16	147	32
Facciuto *et al*[10]	2008	USA	Case-control	5	32	30
Hwang *et al*[11]	2007	Korea	Case-control	17	200	31
Kim *et al*[12]	2008	Korea	Case-control	15	31	30
Margarit *et al*[13]	2005	Spain	Case-control	5	36	31
Sapisochin *et al*[14]	2010	Spain	Case-control	17	34	33
Shao *et al*[15]	2008	China	Case-control	15	62	30
Vennarecci *et al*[16]	2007	Italy	Case-control	9	37	30

SLT: Salvage liver transplantation; PLT: Primary liver transplantation.

Data extraction

All data were extracted independently by 2 reviewers according to the selection criteria. We resolved disagreement through discussion. The following data were extracted: the last name of the first author, study design, publication year, definition of SLT, the type of population described [adults or children (< 18 years)], country of transplant center, number of SLT cases and control (PLT) studies, overall survival, overall recurrence and assessment of risk factors.

Statistical analysis

Meta-analysis was performed using fixed-effect or random-effect methods, depending on the absence or presence of significant heterogeneity. Statistical heterogeneity between trials was evaluated by the Cochran χ^2 test and was considered significant when $P < 0.10$. In the absence of statistically significant heterogeneity, the Mantel-Haenszel method in the fixed-effect model was used for the meta-analysis. Otherwise, the DerSimonian and Laird method in the random-effect model was selected.

The OR with 95% CI was used to assess treatment efficacy. The combined result was an average OR and 95% CI weighted according to the standard error of the OR of the trial. $P < 0.05$ was considered statistically significant. We used funnel plots to assess the publication bias, and tested for funnel plot asymmetry using Egger's test and Begg's test. All analyses were performed with Review Manager version 5.0.23 (RevMan, Cochrane Collaboration, Oxford, England).

RESULTS

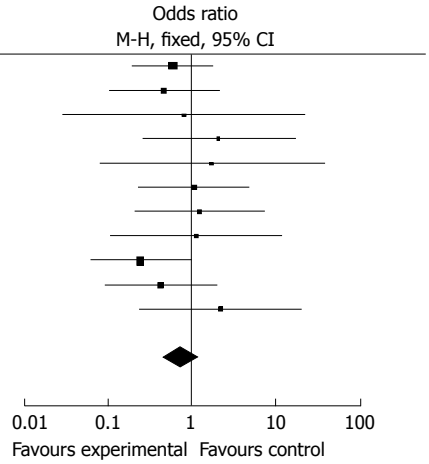
Studies included in the meta-analysis

There were 410 papers relevant to the search words. Via steps of screening the title, abstract reviewing and article reviewing, 11 studies which included 141 SLT cases and 872 PLT cases were identified to match our inclusion criteria[6-16]. Studies had been carried out in France, Italy, USA, China, Spain, Korea and Chinese Taiwan. Details of studies and the methodological quality of the studies assessed according to a score system described above are described in Table 1.

A Review: SLT *vs* PLT; Comparison: SLT *vs* PLT; Outcome: 1-year survival rates

Study or subgroup	SLT Events	SLT Total	PLT Events	PLT Total	Weight %	Odds ratio M-H, fixed, 95% CI
Adam *et al*[6]	12	17	156	195	20.9	0.60 (0.20, 1.80)
Belghiti *et al*[7]	15	18	64	70	12.4	0.47 (0.11, 2.09)
Concejero *et al*[8]	7	7	27	28	2.1	0.82 (0.03, 22.20)
Del Gaudio *et al*[9]	15	16	129	147	4.5	2.09 (0.26, 16.81)
Facciuto *et al*[10]	5	5	28	32	2.1	1.74 (0.08, 37.08)
Hwang *et al*[11]	15	17	175	200	9.2	1.07 (0.23, 4.97)
Kim *et al*[12]	13	15	26	31	6.4	1.25 (0.21, 7.34)
Margarit *et al*[13]	4	5	28	36	3.9	1.14 (0.11, 11.72)
Sapisochin *et al*[14]	10	17	29	34	22.6	0.25 (0.06, 0.95)
Shao *et al*[15]	12	15	56	62	12.4	0.43 (0.09, 1.96)
Vennarecci *et al*[16]	8	9	29	37	3.6	2.21 (0.24, 20.35)
Total (95% CI)		141		872	100.0	0.74 (0.46, 1.21)
Total events	116		747			

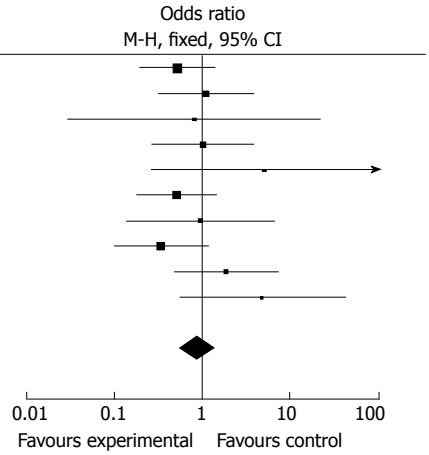
Heterogeneity: χ^2 = 6.42, df = 10 (P = 0.78), I^2 = 0%
Test for overall effect: Z = 1.20 (P = 0.23)



B Review: SLT *vs* PLT; Comparison: SLT *vs* PLT; Outcome: 3-year survival rates

Study or subgroup	SLT Events	SLT Total	PLT Events	PLT Total	Weight %	Odds ratio M-H, fixed, 95% CI
Adam *et al*[6]	9	17	133	195	22.9	0.52 (0.19, 1.42)
Belghiti *et al*[7]	14	18	53	70	11	1.12 (0.33, 3.87)
Concejero *et al*[8]	7	7	27	28	1.7	0.82 (0.03, 22.20)
Del Gaudio *et al*[9]	13	16	119	147	10	1.02 (0.27, 3.82)
Facciuto *et al*[10]	5	5	22	32	1.3	5.13 (0.26, 101.70)
Hwang *et al*[11]	11	17	156	200	19.7	0.52 (0.18, 1.48)
Margarit *et al*[13]	3	5	22	36	4.9	0.95 (0.14, 6.45)
Sapisochin *et al*[14]	9	17	26	34	18.6	0.35 (0.10, 1.19)
Shao *et al*[15]	12	15	42	62	7.5	1.90 (0.48, 7.52)
Vennarecci *et al*[16]	8	9	23	37	2.3	4.87 (0.55, 43.18)
Total (95% CI)		126		841	100.0	0.89 (0.58, 1.37)
Total events	91		623			

Heterogeneity: χ^2 = 9.37, df = 9 (P = 0.40), I^2 = 4%
Test for overall effect: Z = 0.51 (P = 0.61)



C Review: SLT *vs* PLT; Comparison: SLT *vs* PLT; Outcome: 5-year survival rates

Study or subgroup	SLT Events	SLT Total	PLT Events	PLT Total	Weight %	Odds ratio M-H, fixed, 95% CI
Adam *et al*[6]	7	17	119	195	23.8	0.45 (0.16, 1.22)
Belghiti *et al*[7]	10	18	37	70	14.3	1.11 (0.39, 3.16)
Concejero *et al*[8]	7	7	25	28	1.5	2.06 (0.10, 44.48)
Del Gaudio *et al*[9]	10	16	107	147	16.7	0.62 (0.21, 1.83)
Hwang *et al*[11]	9	17	144	200	22.5	0.44 (0.16, 1.19)
Margarit *et al*[13]	1	5	11	36	4.5	0.57 (0.06, 5.69)
Sapisochin *et al*[14]	9	17	22	34	14.6	0.61 (0.19, 2.00)
Vennarecci *et al*[16]	8	9	23	37	2.1	4.87 (0.55, 43.18)
Total (95% CI)		106		747	100.0	0.72 (0.46, 1.11)
Total events	61		488			

Heterogeneity: χ^2 = 6.05, df = 7 (P = 0.53), I^2 = 0%
Test for overall effect: Z = 1.50 (P = 0.13)

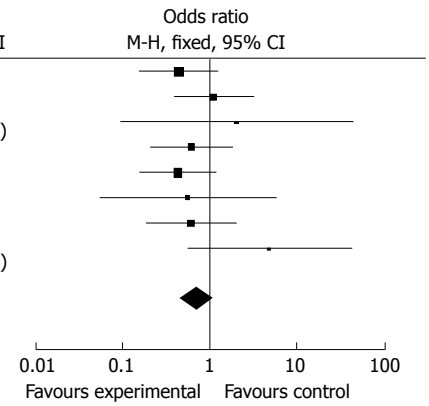


Figure 1 Fixed-effect model of odds ratio for 1-year survival rates (A), 3-year survival rates (B) and 5-year survival rates (C) after salvage liver transplantation and primary liver transplantation (Experimental: Salvage liver transplantation; Control: Primary liver transplantation). SLT: Salvage liver transplantation; PLT: Primary liver transplantation.

Meta-analysis

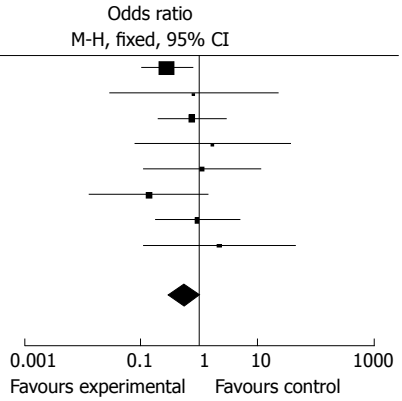
1-year survival after SLT and PLT: A total of 1013 patients were included in 11 articles. According to χ^2 test of heterogeneity (P = 0.78), a fixed-effect model was used. No difference between SLT group (82.3%) and PLT group (85.7%) were seen in the 1-year survival rate (OR: 0.74, 95% CI: 0.46-1.21, P = 0.23, Figure 1A).

3-year survival after SLT and PLT: A total of 967 patients were included in 10 articles. According to χ^2 test of heterogeneity (P = 0.40), a fixed-effect model was used. No difference between SLT group (72.2%) and PLT

A Review: SLT *vs* PLT; Comparison: SLT *vs* PLT; Outcome: 1-year disease-free survival rates

Study or subgroup	SLT Events	SLT Total	PLT Events	PLT Total	Weight %	Odds ratio M-H, fixed, 95% CI
Adam *et al*[6]	8	17	148	195	45.8	0.28 (0.10, 0.77)
Concejero *et al*[8]	7	7	27	28	2.7	0.82 (0.03, 22.20)
Del Gaudio *et al*[9]	13	16	125	147	16.8	0.76 (0.20, 2.90)
Facciuto *et al*[10]	5	5	28	32	2.7	1.74 (0.08, 37.08)
Margarit *et al*[13]	4	5	28	36	5	1.14 (0.11, 11.72)
Sapisochin *et al*[14]	14	17	33	34	14.2	0.14 (0.01, 1.48)
Shao *et al*[15]	13	15	54	62	10.2	0.96 (0.18, 5.08)
Vennarecci *et al*[16]	8	8	33	37	2.6	2.28 (0.11, 46.66)
Total (95% CI)		90		571	100.0	0.56 (0.31, 1.00)
Total events	72		476			

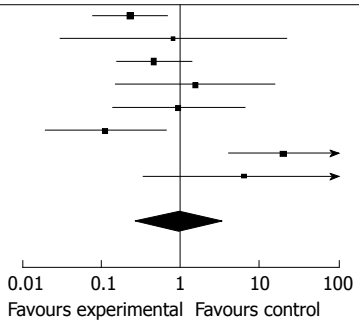
Heterogeneity: χ^2 = 5.48, df = 7 (P = 0.60), I^2 = 0%
Test for overall effect: Z = 1.95 (P = 0.05)



B Review: SLT *vs* PLT; Comparison: SLT *vs* PLT; Outcome: 3-year disease-free survival rates

Study or subgroup	SLT Events	SLT Total	PLT Events	PLT Total	Weight %	Odds ratio M-H, random, 95% CI
Adam *et al*[6]	5	17	125	195	15.8	0.23 (0.08, 0.69)
Concejero *et al*[8]	7	7	27	28	8.1	0.82 (0.03, 22.20)
Del Gaudio *et al*[9]	10	16	115	147	15.8	0.46 (0.16, 1.37)
Facciuto *et al*[10]	4	5	23	32	11.2	1.57 (0.15, 15.97)
Margarit *et al*[13]	3	5	22	36	12.7	0.95 (0.14, 6.45)
Sapisochin *et al*[14]	11	17	32	34	13.4	0.11 (0.02, 0.65)
Shao *et al*[15]	13	15	15	62	13.9	20.37 (4.12, 100.69)
Vennarecci *et al*[16]	8	8	27	37	9.1	6.49 (0.34, 122.71)
Total (95% CI)		90		571	100.0	0.98 (0.27, 3.47)
Total events	61		386			

Heterogeneity: Tau^2 = 2.34; χ^2 = 29.43, df = 7 (P = 0.0001), I^2 = 76%
Test for overall effect: Z = 0.04 (P = 0.97)



C Review: SLT *vs* PLT; Comparison: SLT *vs* PLT; Outcome: 5-year disease-free survival rates

Study or subgroup	SLT Events	SLT Total	PLT Events	PLT Total	Weight %	Odds ratio M-H, random, 95% CI
Adam *et al*[6]	12	17	113	195	25.5	1.74 (0.59, 5.14)
Concejero *et al*[8]	7	7	25	28	7.7	2.06 (0.10, 44.48)
Del Gaudio *et al*[9]	8	16	104	147	26.1	0.41 (0.15, 1.17)
Margarit *et al*[13]	1	5	11	36	11.9	0.57 (0.06, 5.69)
Sapisochin *et al*[14]	10	17	30	34	20.6	0.19 (0.05, 0.79)
Vennarecci *et al*[16]	8	8	27	37	8.3	6.49 (0.34, 122.71)
Total(95% CI)		70		477	100.0	0.75 (0.29, 1.96)
Total events	46		310			

Heterogeneity: Tau^2 = 0.63; χ^2 = 9.81, df = 5 (P = 0.08), I^2 = 49%
Test for overall effect: Z = 0.59 (P = 0.56)

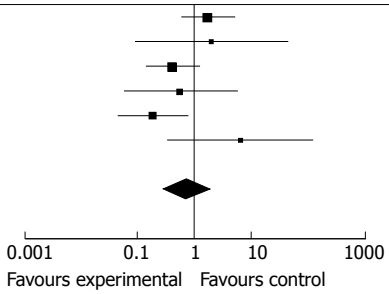


Figure 2 Fixed-effect model of odds ratio for 1-year disease-free survival rates (A); random-effect model of odds ratio for 3-year disease-free survival rates (B) and 5-year disease-free survival rates (C) after salvage liver transplantation and primary liver transplantation (Experimental: Salvage liver transplantation; Control: Primary liver transplantation). SLT: Salvage liver transplantation; PLT: Primary liver transplantation.

group (74.1%) were seen in the 3-year survival rate (OR: 0.89, 95% CI: 0.58-1.37, P = 0.61, Figure 1B).

5-year survival after SLT and PLT: A total of 853 patients were included in 8 articles. According to χ^2 test of heterogeneity (P = 0.53), a fixed-effect model was used. No difference between SLT group (57.5%) and PLT group (65.3%) were seen in the 5-year survival rate (OR: 0.72, 95% CI: 0.46-1.11, P = 0.13, Figure 1C).

1-year disease-free survival after SLT and PLT: A total of 620 patients were included in 8 articles. According to χ^2 test of heterogeneity (P = 0.60), a fixed-effect model was used. No difference between SLT group (80.0%) and PLT group (83.4%) were seen in the 1-year disease-free survival rate (OR: 0.56, 95% CI: 0.31-1.00, P = 0.05, Figure 2A).

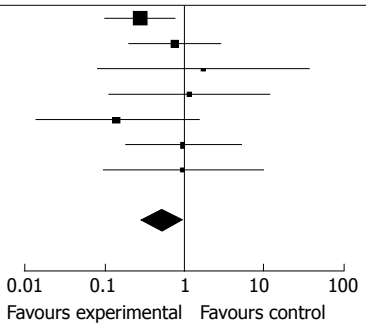
3-year disease-free survival after SLT and PLT: A total of 620 patients were included in 8 articles. According to χ^2 test of heterogeneity (P = 0.0001), a random-effect model was used. No difference between SLT group (67.8%) and PLT group (67.6%) were seen in the 3-year disease-free survival rate (OR: 0.98, 95% CI: 0.27-3.47, P = 0.97, Figure 2B).

5-year disease-free survival after SLT and PLT: A total of 506 patients were included in 6 articles. According

A Review: SLT *vs* PLT; Comparison: SLT *vs* PLT; Outcome: 1-year disease-free survival rates for DDLT recipients

Study or subgroup	SLT Events	SLT Total	PLT Events	PLT Total	Weight %	Odds ratio M-H, fixed, 95% CI
Adam *et al*[6]	8	17	148	195	45.9	0.28 (0.10, 0.77)
Del Gaudio *et al*[9]	13	16	125	147	16.8	0.76 (0.20, 2.90)
Facciuto *et al*[10]	5	5	28	32	2.7	1.74 (0.08, 37.08)
Margarit *et al*[13]	4	5	28	36	5	1.14 (0.11, 11.72)
Sapisochin *et al*[14]	14	17	33	34	14.2	0.14 (0.01, 1.48)
Shao *et al*[15]	13	15	54	62	10.2	0.96 (0.18, 5.08)
Vennarecci *et al*[16]	8	9	33	37	5.2	0.97 (0.09, 9.90)
Total (95% CI)		84		543	100.0	0.53 (0.29, 0.95)
Total events	65		449			

Heterogeneity: χ^2 = 4.76, df = 6 (P = 0.58), I^2 = 0%
Test for overall effect: Z = 2.12 (P = 0.03)



B Review: SLT *vs* PLT; Comparison: SLT *vs* PLT; Outcome: 5-year disease-free survival rates for DDLT recipients

Study or subgroup	SLT Events	SLT Total	PLT Events	PLT Total	Weight %	Odds ratio M-H, fixed, 95% CI
Adam *et al*[6]	5	17	113	195	37	0.30 (0.10, 0.89)
Del Gaudio *et al*[9]	8	16	104	147	29.5	0.41 (0.15, 1.17)
Margarit *et al*[13]	1	5	11	36	6.2	0.57 (0.06, 5.69)
Sapisochin *et al*[14]	10	17	30	34	23.8	0.19 (0.05, 0.79)
Vennarecci *et al*[16]	8	9	27	37	3.4	2.96 (0.33, 26.79)
Total (95% CI)		64		449	100.0	0.42 (0.23, 0.74)
Total events	32		285			

Heterogeneity: χ^2 = 4.62, df = 4 (P = 0.33), I^2 = 13%
Test for overall effect: Z = 2.96 (P = 0.003)

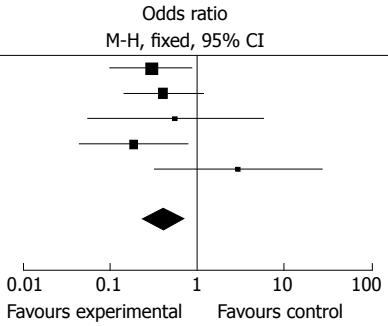


Figure 3 Fixed-effect model of odds ratio for 1-year (A) and 5-year (B) disease-free survival rates for deceased-donor liver transplantation recipients after salvage liver transplantation and primary liver transplantation (Experimental: Salvage liver transplantation; Control: Primary liver transplantation). DDLT: Deceased-donor liver transplantation; SLT: Salvage liver transplantation; PLT: Primary liver transplantation.

to χ^2 test of heterogeneity (P = 0.08), a random-effect model was used. No difference between SLT group (65.7%) and PLT group (65.0%) were seen in the 5-year disease-free survival rate (OR: 0.75, 95% CI: 0.29-1.96, P = 0.56, Figure 2C).

When stratifying for the donor source, compared with deceased-donor liver transplantation (DDLT) recipients, we found that living-donor liver transplantation (LDLT) recipients had significantly higher 1-year survival rate (OR: 1.02, 95% CI: 0.26-4.10, P = 0.97), lower 3-year survival rate (OR: 0.54, 95% CI: 0.20-1.47, P = 0.23) and lower 5-year survival rate (OR: 0.54, 95% CI: 0.21-1.35, P = 0.19). DDLT recipients had significantly lower 1-year survival rate (OR: 0.66, 95% CI: 0.38-1.15, P = 0.14), higher 3-year survival rate (OR: 0.99, 95% CI: 0.62-1.59, P = 0.97) and higher 5-year survival rate (OR: 0.77, 95% CI: 0.47-1.26, P = 0.30). No useable data about disease-free survival rates can be extracted from LDLT researches. And in DDLT recipients they had better 1-year disease-free survival rate (OR: 0.53, 95% CI: 0.29-0.95, P = 0.03, Figure 3A) and better 5-year disease-free survival rate (OR: 0.42, 95% CI: 0.23-0.74, P = 0.003, Figure 3B) in SLT group. No difference between SLT group and PLT group were seen in the 3-year disease-free survival rate (OR: 0.95, 95% CI: 0.26-3.52, P = 0.94).

When stratifying for Milan criteria, we found that no difference was seen in 1-year survival rates (OR: 0.26, 95% CI: 0.01-4.94, P = 0.37), 3-year survival rates (OR: 0.41, 95% CI: 0.01-24.54, P = 0.67) and 5-year survival rates (OR: 0.55, 95% CI: 0.07-4.48, P = 0.57) between SLT group and PLT group who beyond Milan criteria at the time of liver transplantation (LT). No usable data for patients who met Milan criteria at the time of LT.

Publication bias

Publication bias may exist when no significant findings remain unpublished, thus artificially inflating the apparent magnitude of an effect. Funnel plots of our study results are shown in Figure 4. The funnel plots on survival and disease-free survival following SLT or PLT for the treatment of HCC showed basic symmetry, which suggested no publication bias.

DISCUSSION

As one of the radical treatments for HCC, LT is nowadays limited by organ shortage. Due to the prolonged waiting times before transplantation, tumor progression and deterioration of liver function may counteract its benefit[2]. The outcome of liver resection is mainly influenced by a high rate of recurrence that limits long-term survival rates. But previous research noted that most of patients with recurrence after primary liver resection were still eligible for LT[2]. Hence, hepatectomy and LT should be considered as complementary, not competitive, treatments for HCC in cirrhotic patients with well-preserved

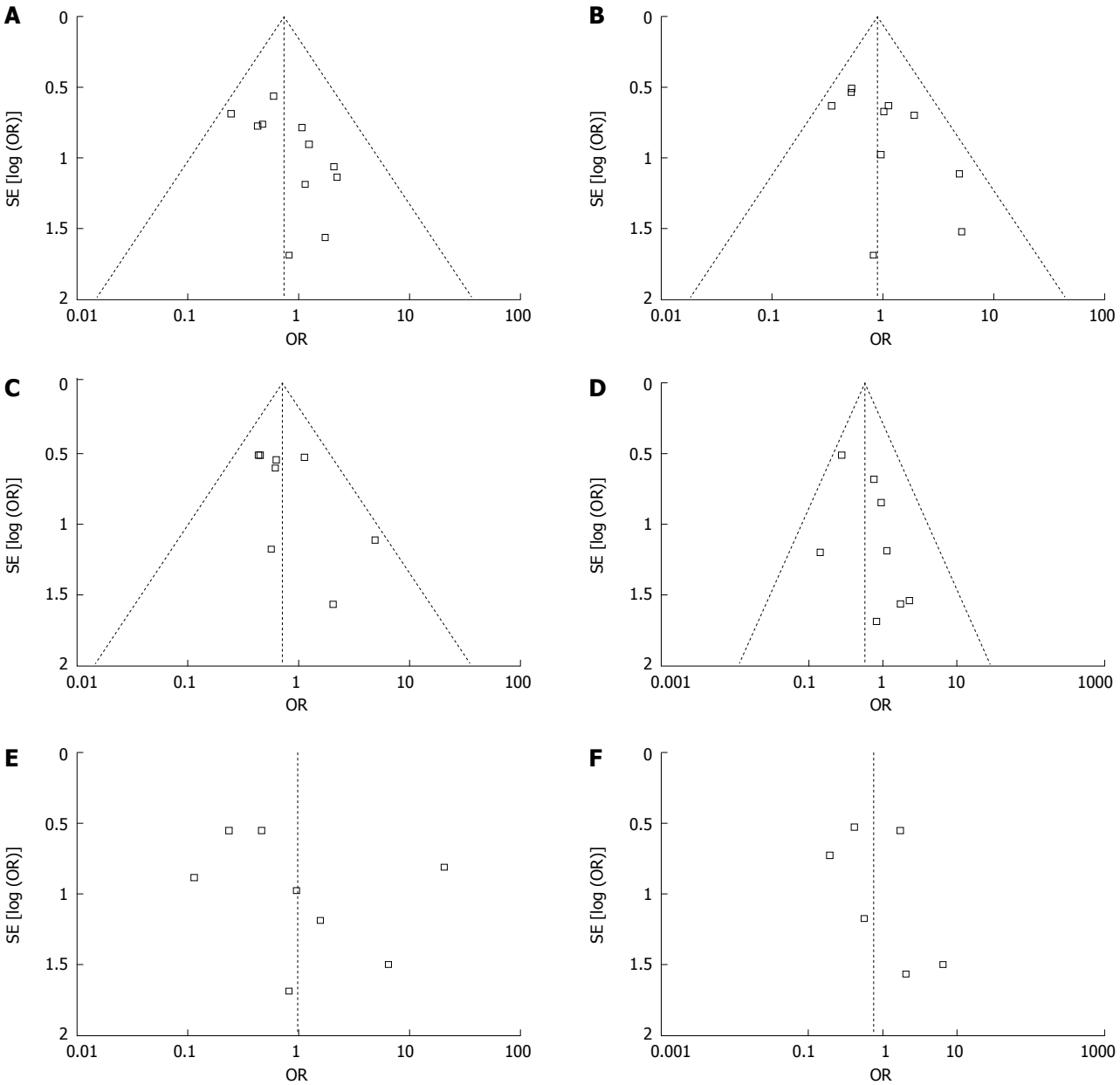


Figure 4 Funnel plot. A: 11 articles in the meta-analysis of 1-year survival after treatment; B: 10 articles in the meta-analysis of 3-years survival after treatment; C: 8 articles in the meta-analysis of 5-years survival after treatment; D: 8 articles in the meta-analysis of 1-year disease-free survival after treatment; E: 8 articles in the meta-analysis of 3-year disease-free survival after treatment; F: 6 articles in the meta-analysis of 5-year disease-free survival after treatment. SE: Standard error; OR: Odds ratio.

liver function. Resection of the liver tumor is an optional bridge treatment[17-19]. SLT was proposed in order to reduce the impact of a long waiting times, donor shortage and tumor recurrence after resection in HCC patients.

The increased technical difficulty during SLT and the risk for impaired posttransplant survival worried most of surgeons. Heavy adhesions and portal hypertension are often encountered after prior liver resection. Inattentive dissection of perihepatic adhesions could result in uncontrollable bleeding at the dissection surface. Also due to heavy adhesions, the relationship between hepatic vein and inferior vena cava are hard to identify. Hwang *et al*[11] found that SLT did not increase the operative risks or postoperative complications. The two major technical concerns-bleeding and reconstruction of the hepatic vein outflow can be solved successfully by steady and meticulous sharp dissection and sufficient dissection of the recipient inferior vena cava. Kim *et al*[12] showed that end-to-end anastomosis for bile ducts and hepatic artery was feasible, too. Our study showed that SLT had no bad effect on overall survival and disease-free survival in comparison with PLT.

Considering different surgical methods may have an effect on survival rates, the whole patients were stratified to LDLT recipients and DDLT recipients. In each subgroup, no difference between SLT group and PLT group were seen in 1-year survival rates, 3-year survival rates and 5-year survival rates. But LDLT recipients may have

a significant higher 1-year survival rates than DDLT recipients, while a significant lower 3-year survival rates and 5-year survival rates. This may be a result of improvement of surgical technique and perioperative management. However, because of a relatively higher incidence (up to 30%) of biliary complication after LDLT[20-23], LDLT recipients may have a lower long-term survival rates than DDLT recipients. Different results in DDLT recipients' disease-free survival rates seem hard to explain. We consider a better tumor stage at the time of transplantation (meet Milan criteria) may contribute to better 1-year disease-free survival rates and 5-year disease-free survival rates. A lager sample and more randomized controlled studies may resolve this conflict and draw a right conclusion.

The tumors' stage at the time of resection and LT is another risk factor for postoperative overall survival and disease-free survival. Some studies were theoretical and assessed the salvage transplantability according to the pattern of recurrence after resection for HCC within Milan criteria and found that 76% to 87% of recurrences were considered eligible for SLT on imaging grounds[2,12,24,25]. For HCC patients not meeting Milan criteria, SLT could be applied for those cases with less aggressiveness, namely tumor size less than 6 cm and pathological well differentiation. For those cases meeting Milan criteria, PLT seems to be the first option. SLT could be performed for those patients with recurrence within Milan criteria after primary resection and without delay before recurrence with advanced disease manifestations. But there is no consensus about the survival rates for patients with recurrence beyond Milan criteria. Our result reveals that SLT group has similar survival rates compared with PLT group beyond Milan criteria at the time of LT. Unfortunately, data extracted from our including studies are not enough to do further meta-analysis on patients' survival rates meeting Milan criteria at the time of LT and the corresponding disease-free survival rates.

Moreover, in countries with a higher incidence of HCC, a higher proportion of HCC patients on the waiting list and/or a longer median time-to-transplant, SLT could offer a gain in life-expectancy to the remaining waiting-list patients[26].

This review has some limitations. Although funnel plots may be suggestive of publication bias with lack of negative small RCTs, a firm conclusion about bias is difficult to make as the asymmetry of the funnel plots is minimal. And funnel plots can show asymmetry for other reasons. Therefore, our pooled OR might be an overestimate of the true effect. Due to data constraints, this meta-analysis could not analyze the quality of life score and was unable to carry out stratified analyses of other possible confounding factors. The method need to be more effective. Larger samples and randomized controlled studies with longer follow-up are required. Our conclusions also need more detailed data to confirm the results. The search language was limited. The integrity of the data was affected to a certain extent.

In conclusion, this new strategy SLT can be effectively performed for patients with recurrence or deterioration of liver function after hepatectomy for HCC. It does not increase the perioperative mortality and has a similar long-term survival rates compared to PLT. When surgical technique is no longer a problem for SLT, more patients will benefit from it.

COMMENTS

Background

Due to shortage of available donors, salvage liver transplantation (SLT) has been proposed and performed for the patients who undergo primary liver resection for hepatocellular carcinoma (HCC) or HCC recurrence or deterioration of liver function. This meta-analysis was designed to evaluated survival and recurrence after SLT for the treatment of HCC compared with primary liver transplantation (PLT).

Research frontiers

The study evaluated survival and recurrence after SLT for the treatment of HCC compared with PLT using a meta-analysis of all relevant controlled studies.

Innovations and breakthroughs

This is the first systematic review and meta-analysis on the survival and recurrence after SLT for the treatment of HCC compared with PLT. The author made a comprehensive search of studies. Several important conclusions might be used for future selection in SLT or PLT for HCC patients' treatments.

Applications

This meta-analysis shows that SLT has a similar survival rates in comparison with PLT. SLT offers an alternative treatment method for HCC patients in facing a shortage of available donors.

Terminology

SLT was defined as a liver transplantation performed for recurrent HCC or deterioration of liver function after primary liver resection.

Peer review

The article should be published as it is a nice overview on the topic after some revisions are performed.

REFERENCES

1 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699

2 **Poon RT**, Fan ST, Lo CM, Liu CL, Wong J. Long-term survival and pattern of recurrence after resection of small hepatocellular carcinoma in patients with preserved liver function: implications for a strategy of salvage transplantation. *Ann Surg* 2002; **235**: 373-382

3 **Chalmers TC**, Smith H, Blackburn B, Silverman B, Schroeder B, Reitman D, Ambroz A. A method for assessing the quality of a randomized control trial. *Control Clin Trials* 1981; **2**: 31-49

4 **Jain A**, Mohanka R, Orloff M, Abt P, Kashyap R, Cullen J, Lansing K, Bozorgzadeh A. University of Wisconsin versus histidine-tryptophan-ketoglutarate for tissue preservation in live-donor liver transplantation. *Exp Clin Transplant* 2006; **4**: 451-457

5 **Stahl JE**, Kreke JE, Malek FA, Schaefer AJ, Vacanti J. Consequences of cold-ischemia time on primary nonfunction and patient and graft survival in liver transplantation: a meta-analysis. *PLoS One* 2008; **3**: e2468

6 **Adam R**, Azoulay D, Castaing D, Eshkenazy R, Pascal G, Hashizume K, Samuel D, Bismuth H. Liver resection as a bridge to transplantation for hepatocellular carcinoma on cirrhosis: a reasonable strategy? *Ann Surg* 2003; **238**: 508-518; discussion 518-519

7 **Belghiti J**, Cortes A, Abdalla EK, Régimbeau JM, Prakash K, Durand F, Sommacale D, Dondero F, Lesurtel M, Sauvanet A, Farges O, Kianmanesh R. Resection prior to liver transplantation for hepatocellular carcinoma. *Ann Surg* 2003; **238**: 885-892; discussion 892-893

8 **Concejero A**, Chen CL, Wang CC, Wang SH, Lin CC, Liu YW, Yang CH, Yong CC, Lin TS, Jawan B, Huang TL, Cheng YF, Eng HL. Living donor liver transplantation for hepatocellular carcinoma: a single-center experience in Taiwan. *Transplantation* 2008; **85**: 398-406

9 **Del Gaudio M**, Ercolani G, Ravaioli M, Cescon M, Lauro A, Vivarelli M, Zanello M, Cucchetti A, Vetrone G, Tuci F, Ramacciato G, Grazi GL, Pinna AD. Liver transplantation for recurrent hepatocellular carcinoma on cirrhosis after liver resection: University of Bologna experience. *Am J Transplant* 2008; **8**: 1177-1185

10 **Facciuto ME**, Koneru B, Rocca JP, Wolf DC, Kim-Schluger L, Visintainer P, Klein KM, Chun H, Marvin M, Rozenblit G, Rodriguez-Davalos M, Sheiner PA. Surgical treatment of hepatocellular carcinoma beyond Milan criteria. Results of liver resection, salvage transplantation, and primary liver transplantation. *Ann Surg Oncol* 2008; **15**: 1383-1391

11 **Hwang S**, Lee SG, Moon DB, Ahn CS, Kim KH, Lee YJ, Ha TY, Song GW. Salvage living donor liver transplantation after prior liver resection for hepatocellular carcinoma. *Liver Transpl* 2007; **13**: 741-746

12 **Kim BW**, Park YK, Kim YB, Wang HJ, Kim MW. Salvage liver transplantation for recurrent hepatocellular carcinoma after liver resection: feasibility of the Milan criteria and operative risk. *Transplant Proc* 2008; **40**: 3558-3561

13 **Margarit C**, Escartín A, Castells L, Vargas V, Allende E, Bilbao I. Resection for hepatocellular carcinoma is a good option in Child-Turcotte-Pugh class A patients with cirrhosis who are eligible for liver transplantation. *Liver Transpl* 2005; **11**: 1242-1251

14 **Sapisochin G**, Bilbao I, Balsells J, Dopazo C, Caralt M, Lázaro JL, Castells L, Allende H, Charco R. Optimization of liver transplantation as a treatment of intrahepatic hepatocellular carcinoma recurrence after partial liver resection: experience of a single European series. *World J Surg* 2010; **34**: 2146-2154

15 **Shao Z**, Lopez R, Shen B, Yang GS. Orthotopic liver transplantation as a rescue operation for recurrent hepatocellular carcinoma after partial hepatectomy. *World J Gastroenterol* 2008; **14**: 4370-4376

16 **Vennarecci G**, Ettorre GM, Antonini M, Santoro R, Maritti M, Tacconi G, Spoletini D, Tessitore L, Perracchio L, Visco G, Puoti C, Santoro E. First-line liver resection and salvage liver transplantation are increasing therapeutic strategies for patients with hepatocellular carcinoma and child a cirrhosis. *Transplant Proc* 2007; **39**: 1857-1860

17 **Otto G**, Heuschen U, Hofmann WJ, Krumm G, Hinz U, Herfarth C. Survival and recurrence after liver transplantation versus liver resection for hepatocellular carcinoma: a retrospective analysis. *Ann Surg* 1998; **227**: 424-432

18 **Pichlmayr R**, Weimann A, Oldhafer KJ, Schlitt HJ, Tusch G, Raab R. Appraisal of transplantation for malignant tumours of the liver with special reference to early stage hepatocellular carcinoma. *Eur J Surg Oncol* 1998; **24**: 60-67

19 **Yamamoto J**, Iwatsuki S, Kosuge T, Dvorchik I, Shimada K, Marsh JW, Yamasaki S, Starzl TE. Should hepatomas be treated with hepatic resection or transplantation? *Cancer* 1999; **86**: 1151-1158

20 **Soejima Y**, Taketomi A, Yoshizumi T, Uchiyama H, Harada N, Ijichi H, Yonemura Y, Ikeda T, Shimada M, Maehara Y. Biliary strictures in living donor liver transplantation: incidence, management, and technical evolution. *Liver Transpl* 2006; **12**: 979-986

21 **Tsujino T**, Isayama H, Sugawara Y, Sasaki T, Kogure H, Nakai Y, Yamamoto N, Sasahira N, Yamashiki N, Tada M, Yoshida H, Kokudo N, Kawabe T, Makuuchi M, Omata M. Endoscopic management of biliary complications after adult living donor liver transplantation. *Am J Gastroenterol* 2006; **101**: 2230-2236

22 **Gondolesi GE**, Varotti G, Florman SS, Muñoz L, Fishbein TM, Emre SH, Schwartz ME, Miller C. Biliary complications in 96 consecutive right lobe living donor transplant recipients. *Transplantation* 2004; **77**: 1842-1848

23 **Liu CL**, Lo CM, Chan SC, Fan ST. Safety of duct-to-duct biliary reconstruction in right-lobe live-donor liver transplantation without biliary drainage. *Transplantation* 2004; **77**: 726-732

24 **Tanaka S**, Noguchi N, Ochiai T, Kudo A, Nakamura N, Ito K, Kawamura T, Teramoto K, Arii S. Outcomes and recurrence of initially resectable hepatocellular carcinoma meeting milan criteria: Rationale for partial hepatectomy as first strategy. *J Am Coll Surg* 2007; **204**: 1-6

25 **Majno PE**, Sarasin FP, Mentha G, Hadengue A. Primary liver resection and salvage transplantation or primary liver transplantation in patients with single, small hepatocellular carcinoma and preserved liver function: an outcome-oriented decision analysis. *Hepatology* 2000; **31**: 899-906

26 **Cucchetti A**, Vitale A, Gaudio MD, Ravaioli M, Ercolani G, Cescon M, Zanello M, Morelli MC, Cillo U, Grazi GL, Pinna AD. Harm and benefits of primary liver resection and salvage transplantation for hepatocellular carcinoma. *Am J Transplant* 2010; **10**: 619-627

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BRIEF ARTICLE

Reduced Popdc3 expression correlates with high risk and poor survival in patients with gastric cancer

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Abstract

AIM: To investigate the expression of Popeye domain containing 3 (Popdc3) and its correlation with clinicopathological features and prognosis of gastric cancer.

METHODS: The method of immunohistochemistry was used to investigate the expression of Popdc3 in 306 cases of human gastric cancer and 84 noncancerous gastric tissues. Simultaneously, the relationship between Popdc3 expression and the survival of the patients was retrospectively analyzed.

RESULTS: Popdc3 was detected in 72 (85.71%) of 84 human nontumor mucosa. High expression of Popdc3 protein was detected in 78 (25.49%) of 306 human gastric cancer cases, and low expression was detected in 228 (74.51%). Low expression of Popdc3 correlated with depth of invasion ($P < 0.0001$), regional lymph nodes ($P < 0.0001$) and distant metastasis ($P = 0.02$), and tumor, nodes, metastasis (TNM) stages ($P < 0.0001$). On multivariate analysis, only the patient's gender, regional lymph node metastasis, distant metastasis, TNM stages, and the expression of Popdc3 were independent prognostic factors in patients with gastric cancer. The Kaplan-Meier plot showed that low Popdc3 expression had a much more significant effect on the survival of those patients with early-stage tumors ($\chi^2 = 104.741$, $P < 0.0001$), with a > 51.9% reduction in the three-year survival compared with high Popdc3 expression. In late stages, the difference was also significant ($\chi^2 = 5.930$, $P = 0.015$), with a 32.6% reduction in the three-year survival.

CONCLUSION: Reduced expression of Popdc3 may play a significant role in the carcinogenesis and progression of gastric cancer. Popdc3 may be an independent prognostic factor.

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Key words: Popeye domain containing 3; Gastric cancer; Cell adhesion molecules; Metastasis; Prognosis

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INTRODUCTION

Gastric cancer is the second leading cause of cancer-related deaths worldwide because advanced or metastatic gastric cancer constitutes the majority of patients in clinical practice[1]. The incidence of gastric cancer has been falling globally in terms of both incidence and mortality rates since World War Ⅱ. In the United States in 2009, 21 130 new diagnoses of gastric cancer were estimated and 10 620 deaths expected[2]. Although there has been a noticeable decrease in gastric cancer mortality rate, there is a higher prevalence of gastric cancer in China than in the Western countries[3-7]. The prognosis of advanced gastric cancer is apparently poor, and the 5-year survival rate is less than 30% in the patients after surgery[8,9]. Therefore, it is of great clinical value to further understand the molecular mechanisms involved in gastric cancer and to find valuable diagnostic markers as well as novel therapeutic strategies.

The Popeye domain containing (Popdc) gene family consists of Bves/Popdc1, Popdc2 and Popdc3[10]. They were discovered in 1999 by two independent laboratories using screens to identify novel genes that were highly expressed in the developing heart[11,12]. Several studies have illuminated that within one species, Bves was 24% and 28% identical to Popdc2 and Popdc3, respectively, while Popdc2 and Popdc3 were approximately 50% identical[13-15]. In 2004, the expression pattern of Popdc2 in the heart of chick was reported, but no study had been conducted to test the function of this protein[14]. Until now, Bves is still the most studied member of the Popdc family[10]. After that, Kim *et al*[16] found that frequent silencing of Popdc3 was associated with promoter hypermethylation in gastric cancer. Although questions about Popdc3 remain unanswered, general trends are beginning to emerge. Previous studies have led us to the hypothesis that Popdc3 may play a role in cell adhesion, cell motility, DNA methylation and tumorigenesis. Nevertheless, further work is required to elucidate its molecular mechanisms in the biology of cancer.

As far as we know, no report is available on the actual expression level of Popdc3 and the correlation between clinicopathologic features and prognosis of gastric cancer patients. Therefore, in this study, we investigated the Popdc3 protein expression profile in 306 primary gastric cancer patients, and found that Popdc3 was lowly expressed in 228 (74.51%) of 306 human gastric cancer cases, suggesting that the low expression level of Popdc3 may be a reliable indicator for the poor prognosis of gastric cancer patients.

MATERIALS AND METHODS

Patients and tissue samples

A total of 306 patients with primary gastric cancer, who underwent routine surgery at the Department of Surgery, the Second Affiliated Hospital of Kunming Medical University from February 1996 to March 2007, were enrolled in this study. The study was approved by the hospital's ethics committee. Patient diagnosis was established pathologically, and no patient had received any treatment before admission. All patients had follow-up records for over 5 years. The follow-up deadline was April 2011. The survival time was counted from the date of surgery to the end of the follow-up or date of death, which was mostly caused by recurrence or metastasis. According to the tumor, nodes, metastasis (TNM)-7th edition 2009 (UICC/AJCC) and Japanese Classification 2010 in Gastric Cancer[17,18], there were 8 papillary adenocarcinomas, 209 tubular adenocarcinomas, 52 mucinous adenocarcinomas, 37 signet ring cell carcinomas, and 17 highly differentiated adenocarcinomas; 100 were classified as well or moderately differentiated adenocarcinomas, 177 as poorly differentiated adenocarcinomas, and 12 as undifferentiated adenocarcinomas or others. Seventy-three cases were categorized as stage Ⅰ, 109 were stage Ⅱ, 92 were stage Ⅲ, and 32 were stage Ⅳ. Eighty-four cases of noncancerous human gastric tissues were obtained from gastrectomies of adjacent gastric cancer margins greater than 5 cm and served as controls.

Immunohistochemistry

Immunohistochemical analysis was undertaken to study altered protein expression in 84 cases of noncancerous human gastric tissues and 306 cases of human gastric cancer tissues[19,20]. According to the protocol for immunohistochemistry on paraffin-embedded tissue sections, slides were baked at 60 ℃ for 2 h followed by deparaffinization with xylene and rehydrated. The sections were submerged into ethylene diamine tetraacetic acid antigenic retrieval buffer and microwaved for antigenic retrieval, after which they were treated with 3% hydrogen peroxide in methanol to block endogenous peroxidase activity, followed by incubation with 1% bovine serum albumin to block nonspecific binding. Sections were incubated with rabbit anti-Popdc3 polyclonal antibody (ProteinTech Group, Chicago IL) overnight at 4 ℃. Normal goat serum was used as a negative control. After rinsing twice for 5 min with Tris buffered saline tween-20, tissue sections were treated with secondary antibody in Tris buffered saline solution for 1 h at room temperature, developed with chromogen at room temperature, and observed under microscope. After that, all tissue sections were counterstained with hematoxylin, dehydrated and mounted. The cytoplasm with strong Popdc3 expression was stained as buffy, whereas weak expression was associated with cell membranes.

Assessment of Popdc3 staining in the tissue sections

The degree of immunostaining was reviewed and scored independently by at least two observers based on the proportion of positively stained tumor cells and intensity of staining[21,22]. Tumor cell proportion was scored as follows: 0 (≤ 5% positive tumor cells), 1 (6%-25% positive tumor cells), 2 (26%-50% positive tumor cells), and 3 (>

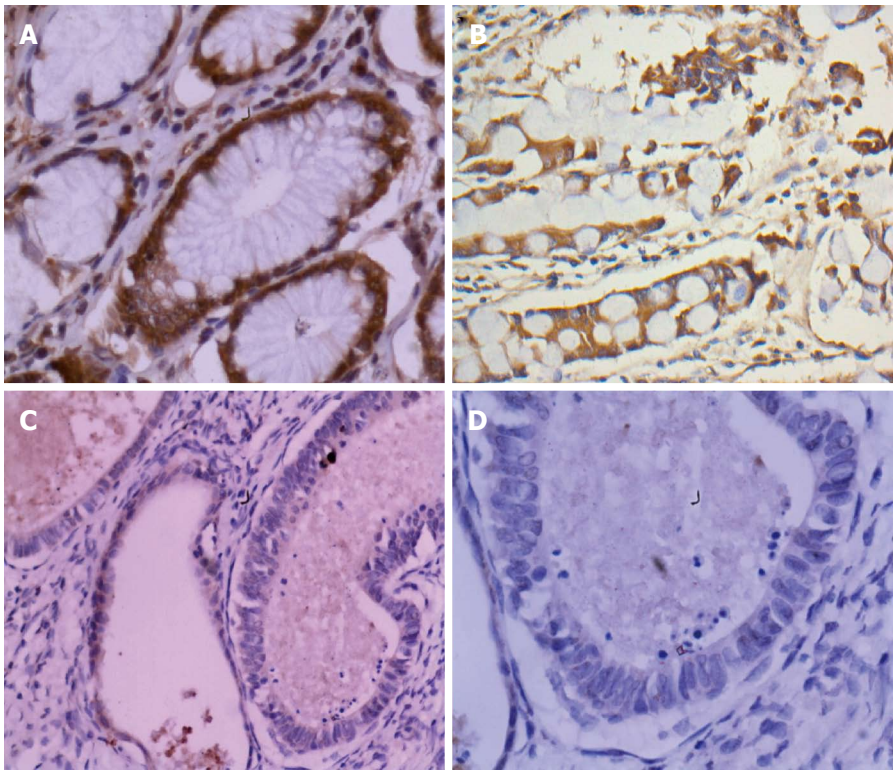

Figure 1 Immunohistochemical staining for Popeye domain containing 3 in gastric cancer lesions and noncancerous tissues. A: Popeye domain containing 3 (Popdc3) was highly expressed in noncancerous tissues, × 400; B: Popdc3 was highly expressed in intestinal metaplasia cells, × 400; C, D: Popdc3 was lowly expressed in tubular adenocarcinoma, × 200 and × 400, respectively.

51% positive tumor cells). Staining intensity was graded according to the following criteria: 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellow brown), and 3 (strong staining, brown). Staining index was calculated as the staining intensity score and the proportion of positive tumor cells. Using this method of assessment, we evaluated Popdc3 expression in benign gastric epithelia and malignant lesions by determining the staining index with scores of 0, 1, 2, 3, 4, 6, or 9. The cutoff value for high and low expression levels was chosen based on the heterogeneity measured using the log-rank test with respect to overall survival. An optimal cutoff value was identified as follows: a staining index score of $\geqslant 4$ was used to define tumors with high Popdc3 expression, and a staining index score of $\leqslant 3$ was used to indicate low expression.

Statistical analysis

All statistical analyses were performed using the SPSS 17.0 software. Correlation of Popdc3 expression with immunohistochemistry and clinicopathologic parameters was evaluated by χ^2 test or Fisher's exact probability test. Overall survival rate was calculated by the Kaplan-Meier method and the difference in survival curves was analyzed by the log-rank test. The follow-up time was calculated from the date of surgery to the date of death, or the last known follow-up. Independent prognostic factors were analyzed by the Cox proportional hazards regression model. $P < 0.05$ was considered statistically significant.

RESULTS

Expression of Popdc3 in gastric cancer and noncancerous mucosa

In our current study, immunohistochemical analysis was done to examine the Popdc3 expression in 306 gastric cancer lesions and 84 noncancerous tissues. Popdc3 was detected in 72 (85.71%) cases of 84 human nontumor mucosa. High expression of Popdc3 protein was detected in 78 (25.49%) cases of 306 human gastric cancer, and low expression was detected in 228 (74.51%). Popdc3 staining was detected mainly in the majority of normal cells, especially in chief cells and smooth muscle. Besides, we also found Popdc3 expression in intestinal metaplasia. However, Popdc3 was mainly localized in the cytoplasm of tumor cells and its weak staining in cell membranes. The differences of Popdc3 expression between gastric cancer and noncancerous mucosa were also statistically significant (χ^2 = 1.010E2, $P < 0.0001$, Figure 1).

Reduced Popdc3 expression and clinicopathologic features

Reduced expression of Popdc3 correlated with depth of invasion, regional lymph node and distant metastasis, and TNM stages ($P < 0.05$). Popdc3 expression did not correlate with age, gender, location of tumor, size of tumor, histologic type and histologic differentiation ($P > 0.05$, Table 1). The factors for possible prognostic effects in gastric cancer were analyzed by Cox regression analysis.

Table 1 Patient characteristics and Popeye domain containing 3 expression in gastric cancer *n* (%)

Clinical parameters	*n*	Popdc3		χ^2	*P* value
		Low	High		
Age (yr)					
< 50	105	72 (68.6)	33 (31.4)		
≥ 50	201	156 (77.6)	45 (22.4)	2.968	0.085
Gender					
Male	199	148 (74.4)	51 (25.6)		
Female	107	80 (74.8)	27 (25.2)	0.006	0.940
Size (cm)					
< 5	154	120 (77.9)	34 (22.1)		
≥ 5	152	108 (71.1)	44 (28.9)	1.901	0.168
Histology					
Papillary adenocarcinoma	8	5 (62.5)	3 (37.5)		
Tubular adenocarcinoma	209	151 (72.2)	58 (27.8)		
Mucinous adenocarcinoma	52	42 (80.8)	10 (19.2)		
Signet ring cell carcinoma	37	30 (81.1)	7 (18.9)	3.084	0.379
Histologic differentiation					
Well	17	13 (76.5)	4 (23.5)		
Moderate	100	67 (67)	33 (33)		
Poor	177	140 (79.1)	37 (20.9)		
Undifferentiated	12	8 (66.7)	4 (33.3)	4.439	0.109
Invasion depth					
T1	24	10 (41.7)	14 (58.3)		
T2	63	32 (50.8)	31 (49.2)		
T3	181	149 (82.3)	32 (17.7)		
T4a	28	24 (85.7)	4 (14.3)		
T4b	10	7 (70.0)	3 (30.0)	48.566	< 0.0001
Regional lymph nodes					
N0	163	94 (57.7)	69 (42.3)		
N1	41	39 (95.1)	2 (4.9)		
N2	54	49 (90.7)	5 (9.3)		
N3a	20	14 (70.0)	6 (30.0)		
N3b	27	19 (70.4)	8 (29.6)	52.504	< 0.0001
Distant metastasis					
M0	274	197 (71.9)	77 (28.1)		
M1	32	31 (96.9)	1 (3.1)	9.412	0.002
TNM stages					
Ⅰ	73	30 (41.1)	43 (58.9)		
Ⅱ	109	81 (74.3)	28 (25.7)		
Ⅲ	92	86 (93.5)	6 (6.5)		
Ⅳ	32	31 (96.9)	1 (3.1)	70.624	< 0.0001

TNM: Tumor, nodes, metastasis; Popdc3: Popeye domain containing 3.

The multivariate analysis suggested that the patient's gender ($P = 0.016$), regional lymph node metastasis ($P < 0.0001$), distant metastasis ($P < 0.0001$), TNM stages ($P = 0.002$), and the expression of Popdc3 ($P < 0.0001$) were independent prognostic factors in patients with gastric carcinoma. However, patient's age, tumor location, size of tumor, histologic type, histologic differentiation and depth of invasion had no prognostic value (Table 2).

Correlation between Popdc3 expression and patient prognosis

In stages Ⅰ and Ⅱ, the 3-year survival rate of patients with a low expression of Popdc3 was significantly lower than in patients with high Popdc3 expression. The survival estimates showed a dramatic difference in median survival between the high and low Popdc3 expression: the former averaged 55 mo [95% confidence interval (CI): 53.515-56.485], whereas the latter 23 mo (95% CI: 21.201-24.799). For patients with low Popdc3 protein expression, 1- and 3-year survival rates were 76.75% and 16.7%, respectively, which were significantly lower than in patients with high Popdc3 expression (88.46%, 84.6%, respectively, $\chi^2 = 145.095$, $P < 0.0001$). Based on this result, we suggested that diminished expression of Popdc3 is a prognostic indicator of poor survival for patients with gastric cancer. In addition, we further compared the survival between the patients in early TNM stage (stages Ⅰ and Ⅱ) or late stages (stages Ⅲ and Ⅳ) with different Popdc3 expression. The results showed that low Popdc3 expression had a much more significant effect on the survival of the patients with early stage tumors ($\chi^2 = 104.741$, $P < 0.0001$), with a > 51.9% reduction in the 3-year survival compared with that of patients with high Popdc3 expression. In late stages, the difference was also significant ($\chi^2 = 5.930$, $P = 0.015$), with a 32.6% reduction in the 3-year survival. Therefore, these data suggested that Popdc3 expression was as an independent prognostic variable for gastric cancer in early stage and late stage (Figure 2 and Table 3).

Table 2 Multivariate analysis for disease-related deaths (Cox regression model)

Variables	*P* value	Hazard ratio	95% CI
Popdc3	< 0.0001	0.193	0.133-0.282
Gender	0.016	1.388	1.063-1.814
Regional lymph nodes	< 0.0001	1.344	1.156-1.564
Distant metastasis	< 0.0001	11.591	6.131-21.915
TNM stages	0.002	1.486	1.159-1.905

CI: Confidence interval; TNM: Tumor, nodes, metastasis; Popdc3: Popeye domain containing 3.

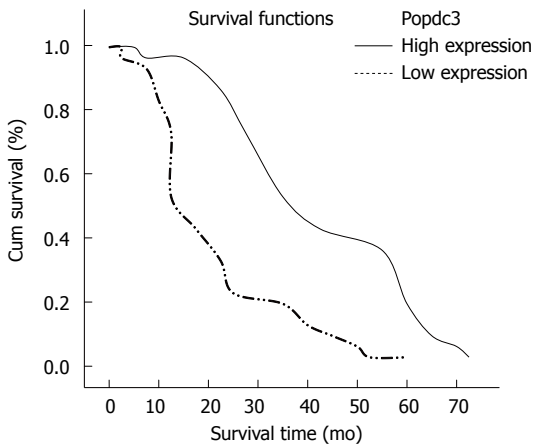

Figure 2 Kaplan-Meier curves with univariate analyses (log-rank) for patients with low Popeye domain containing 3 expression *vs* high Popeye domain containing 3 expression tumors in all gastric cancer. The cumulative and 3-year survival rates were 88.46% and 84.6%, respectively in the high Popeye domain containing 3 (Popdc3) protein expression group, but was only 76.75% and 16.7% in the low expression group ($\chi^2 = 145.095$, $P < 0.0001$).

DISCUSSION

The cause of cancer remains elusive and may be multifac-

Table 3 Survival estimates and 95% confidence intervals for disease-related deaths

Groups	Median survival (mo)	1-yr survival rate (%)	3-yr survival rate (%)	χ^2	*P* value
Low Popdc3 expression	23 (21.20-24.79)	175 (76.75)	38 (16.7)		
High Popdc3 expression	55 (53.51-56.48)	69 (88.46)	66 (84.6)	145.095	< 0.0001
Stage Ⅰ-Ⅱ with low Popdc3 expression	29 (21.00-38.00)	97 (87.39)	44 (39.64)		
Stage Ⅰ-Ⅱ with high Popdc3 expression	55 (45.00-61.00)	65 (91.55)	65 (91.55)	104.741	< 0.0001
Stage Ⅲ-Ⅳ with low Popdc3 expression	15 (10.00-21.50)	78 (66.67)	12 (10.26)		
Stage Ⅲ-Ⅳ with high Popdc3 expression	30 (12.00-44.00)	4 (57.14)	3 (42.86)	5.93	0.015

Popdc3: Popeye domain containing 3.

torial[23,24]. Work by many researchers has illuminated that epigenetic alterations, like mutations and chromosomal abnormalities, can be causally involved in carcinogenesis[25,26]. Previous publications discovered that aberrant DNA methylation was more frequently present in cancers than mutations[27,28].

As a prototypical member of the Popdc family, Bves is mostly studied over the past several decades. Many reports have shown that Bves is highly conserved and has been identified in a wide variety of vertebrates and invertebrates[29,30]. Both mRNA and protein of Bves are highly expressed in striated and smooth muscles and in various forms of epithelial cell types in the embryo and adult[31,32]. It seems that Bves plays a critical role in cell-cell adhesion and in maintaining epithelial integrity, suggesting that loss of Bves function could result in abnormal cell behavior and disease[33-35]. In 2008, Feng *et al*[36] used MethyLight assays to analyze DNA methylation status of 27 genes on 49 paired cancerous and noncancerous tissue samples from non-small cell lung cancer (NSCLC) patients and found that Bves were methylated significantly more frequently in tumor tissues than in noncancerous tissues. Methylation of Bves was present in 80% of NSCLC tissues but only in 14% of noncancerous tissues. It is, so far, the first report of a modification of Bves in cancer.

In 2010, Kim *et al*[16] conducted the first detailed research on the Popdc family and discovered that frequent silencing of Bves and Popdc3 was associated with promoter hypermethylation in gastric cancer. Expression of Bves and Popdc3 was downregulated in 73% of the gastric cancer cell lines and in 69% (Bves) and 87% (Popdc3) of the gastric cancer tissues. Bves and Popdc3 were hypermethylated in 69% (Bves) and 64% (Popdc3) of the gastric cancer tissues. They also found that combined treatment with a DNA methylation inhibitor and a histone deacetylase inhibitor strongly induced Bves and Popdc3 expression. These observations suggested that frequent methylation and inactivation of Bves and Popdc3 in early-stage gastric cancer might predispose cells to other critical changes that cause cancer metastasis.

These results are similar to our study. However, the clinical impact of Popdc3 expression or the prognostic value for gastric cancer was not completely clarified because the number of gastric cancer patients was too small. Actually, it is the first study to explore the correlation between Popdc3 expression and clinical and prognostic factors in gastric cancer. Our study demonstrated that Popdc3 was frequently downregulated in gastric cancer tissues in comparison with those in normal gastric tissues. We examined the relationship between Popdc3 expression and clinicopathological factors in gastric cancer. As a result, reduced level of Popdc3 protein expression in gastric cancer lesions was found mainly associated with depth of invasion, regional lymph node and distant metastasis, and TNM stages. Bves is required for maintenance of E-cadherin in the membrane and plays an important role in cell adhesion and in maintaining epithelial integrity[32]. In development of tumor tissues or diseases, downregulation or mislocalization of E-cadherin is associated with epithelial-mesenchymal transition (EMT)[37]. EMT is considered to be essential for proper development and underlies embryonic processes such as chick gastrulation and coronary vasculature formation[38]. When spontaneously or aberrantly induced in the adult, however, EMT as a hallmark of cancer, may result in loss of epithelial organization and cell tumor tissues invasion of previously normal tissues[39]. Therefore, interfering with E-cadherin function, loss of Bves could result in abnormal cell behavior and disease by promoting EMT programs[40]. Bves was 28% identical to Popdc3 among Popeye family members[12], indicating that Popdc3 may play a role in tumor suppression and interact with Bves.

In this study, multivariate analysis revealed that patient's gender, regional lymph node and distant metastasis, TNM stages, and the expression of Popdc3 were independent prognostic factors for the disease. Although abnormal expression of Popdc3 in gastric cancer might play an important role in the process of tumorigenesis, its biochemical mechanism and potential impact on patient's survival is unknown. So we further analyzed and assessed the impact of expression of Popdc3 on patient's survival. The result indicated that low levels of Popdc3 protein were closely correlated with the prognosis of gastric cancer. A survival curve plotted by the Kaplan-Meier method showed that in patients with low Popdc3 protein expression, the 1- and 3-year survival rates were 76.75% and 16.7%, respectively, which were significantly lower than in patients with high Popdc3 expression (88.46% and 84.6%, respectively). Besides, we further compared the survival between the patients with Popdc3 expression in early TNM stage (stages Ⅰ and Ⅱ) or late stage (stages Ⅲ and Ⅳ). We found that low Popdc3 expression had a much more significant effect on the survival of those patients with early stage tumors, with a > 51.9% reduc-

tion in the 3-year survival as compared with high Popdc3 expression. In late stages, the difference was also significant, with a 32.6% reduction in 3-year survival. These results suggested Popdc3 expression is an independent prognostic variable for gastric cancer in early stage and late stage. In this regard, routine detection of methylation of Popdc3 in blood might be useful in monitoring and detecting tumor recurrence in early-stage gastric cancer after curative surgical resection.

In conclusion, our study suggests that degradation of Popdc3 is a common feature in gastric cancer that might play an important role in the progression and metastases of gastric cancer. In addition, the potentially important consequence of our work is that Popdc3 may be an attractive therapeutic candidate for gastric cancer. Thus, we believe that more researches on Popdc3 will further provide a basis for the development of potential biomarkers for the diagnosis and prognosis of gastric cancer.

COMMENTS

Background

Although many molecular and biological studies have shown risk factors for gastric cancer, the exact molecular mechanism of gastric cancer has not been clarified completely. There is an urgent need to find special markers closely related to tumor outcome and therapy.

Research frontiers

The Popeye domain containing (Popdc) gene family consists of Bves/Popdc1, Popdc2 and Popdc3. As a prototypical member of the Popdc family, Bves is mostly studied over the past decades. However, the clinical impact of Popdc3 expression on cancers is still unknown. This study was carried out to investigate the alterations in the expression of Popdc3 in surgical specimens of gastric cancer, to explore the possible correlation between Popdc3 expression and clinicopathologic variables, to correlate expression of Popdc3 with lymph node metastasis and distant metastasis.

Innovations and breakthroughs

This is the first study attempting to elucidate the role of Popdc3 in gastric cancer and its prognostic significance. The findings suggest that Popdc3 is downregulated in gastric cancer tissues in comparison with those in normal gastric tissues. This study further demonstrates that degradation of Popdc3 is associated with gastric cancer progression and survival of the patients.

Applications

The results will open an avenue for further research to evaluate the role of Popdc3 in gastric cancer. Popdc3 expression may represent a potential prognostic marker and therapeutic target for gastric cancer.

Terminology

Popdc3, as a member of the Popdc family, was firstly detected in developing and adult striated muscle in vertebrates. Chromosomal mapping indicates that Popdc3 gene is clustered on mouse chromosome 10. Frequent methylation and inactivation of Popdc3 are observed in early-stage gastric cancer might predispose cells to other critical changes that cause cancer metastasis.

Peer review

This is a very well written article about the clinical significance of Popdc3 protein expression observed by IHC in gastric cancer. The message of this article was very clear but the author only did IHC to see the expression of Popdc3. This weakened the strength of this article.

REFERENCES

1 **Ajani JA**, Barthel JS, Bekaii-Saab T, Bentrem DJ, D'Amico TA, Das P, Denlinger C, Fuchs CS, Gerdes H, Hayman JA, Hazard L, Hofstetter WL, Ilson DH, Keswani RN, Kleinberg LR, Korn M, Meredith K, Mulcahy MF, Orringer MB, Osarogiagbon RU, Posey JA, Sasson AR, Scott WJ, Shibata S, Strong VE, Washington MK, Willett C, Wood DE, Wright CD, Yang G. Gastric cancer. *J Natl Compr Canc Netw* 2010; **8**: 378-409

2 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249

3 **Ye YW**, Dong RZ, Zhou Y, Du CY, Wang CM, Fu H, Shi YQ. Prognostic analysis of familial gastric cancer in Chinese population. *J Surg Oncol* 2011; **104**: 76-82

4 **Chen JG**, Zhu J, Zhang YH, Lu JH. Cancer survival in Qidong, China, 1992-2000. *IARC Sci Publ* 2011; **(162)**: 43-53

5 **Law SC**, Mang OW. Cancer survival in Hong Kong SAR, China, 1996-2001. *IARC Sci Publ* 2011; **(162)**: 33-41

6 **Xishan H**, Chen K, Min H, Shufen D, Jifang W. Cancer survival in Tianjin, China, 1991-1999. *IARC Sci Publ* 2011; **(162)**: 69-84

7 **Xiang YB**, Jin F, Gao YT. Cancer survival in Shanghai, China, 1992-1995. *IARC Sci Publ* 2011; **(162)**: 55-68

8 **Thun M**, Jemal A, Desantis C, Blackard B, Ward E. An overview of the cancer burden for primary care physicians. *Prim Care* 2009; **36**: 439-454

9 **Wang J,** Yu JC, Kang WM, Ma ZQ. Treatment strategy for early gastric cancer. *Surg Oncol* 2011; Epub ahead of print

10 **Hager HA**, Bader DM. Bves: ten years after. *Histol Histopathol* 2009; **24**: 777-787

11 **Reese DE**, Zavaljevski M, Streiff NL, Bader D. bves: A novel gene expressed during coronary blood vessel development. *Dev Biol* 1999; **209**: 159-171

12 **Andrée B**, Hillemann T, Kessler-Icekson G, Schmitt-John T, Jockusch H, Arnold HH, Brand T. Isolation and characterization of the novel popeye gene family expressed in skeletal muscle and heart. *Dev Biol* 2000; **223**: 371-382

13 **Wada AM**, Reese DE, Bader DM. Bves: prototype of a new class of cell adhesion molecules expressed during coronary artery development. *Development* 2001; **128**: 2085-2093

14 **Breher SS**, Mavridou E, Brenneis C, Froese A, Arnold HH, Brand T. Popeye domain containing gene 2 (Popdc2) is a myocyte-specific differentiation marker during chick heart development. *Dev Dyn* 2004; **229**: 695-702

15 **Vasavada TK**, DiAngelo JR, Duncan MK. Developmental expression of Pop1/Bves. *J Histochem Cytochem* 2004; **52**: 371-377

16 **Kim M**, Jang HR, Haam K, Kang TW, Kim JH, Kim SY, Noh SM, Song KS, Cho JS, Jeong HY, Kim JC, Yoo HS, Kim YS. Frequent silencing of popeye domain-containing genes, BVES and POPDC3, is associated with promoter hypermethylation in gastric cancer. *Carcinogenesis* 2010; **31**: 1685-1693

17 **Santiago JM**, Sasako M, Osorio J. [TNM-7th edition 2009 (UICC/AJCC) and Japanese Classification 2010 in Gastric Cancer. Towards simplicity and standardisation in the management of gastric cancer]. *Cir Esp* 2011; **89**: 275-281

18 **Washington K**. 7th edition of the AJCC cancer staging manual: stomach. *Ann Surg Oncol* 2010; **17**: 3077-3079

19 **Zeng Q**, Zhao Y, Yang Y, Chen XX, Wang G, Zhang P, Cui Y, Su S, Li K. Expression of Cystatin C in human stomach neoplasms. *Mol Med Report* 2010; **3**: 607-611

20 **Ishigami S**, Ueno S, Nishizono Y, Matsumoto M, Kurahara H, Arigami T, Uchikado Y, Setoyama T, Arima H, Yoshiaki K, Kijima Y, Kitazono M, Natsugoe S. Prognostic impact of CD168 expression in gastric cancer. *BMC Cancer* 2011; **11**: 106

21 **Hofmann M**, Stoss O, Shi D, Büttner R, van de Vijver M, Kim W, Ochiai A, Rüschoff J, Henkel T. Assessment of a HER2 scoring system for gastric cancer: results from a validation study. *Histopathology* 2008; **52**: 797-805

22 **Geller SA**, Dhall D, Alsabeh R. Application of immunohistochemistry to liver and gastrointestinal neoplasms: liver, stomach, colon, and pancreas. *Arch Pathol Lab Med* 2008; **132**: 490-499

23 **Nobili S**, Bruno L, Landini I, Napoli C, Bechi P, Tonelli F, Rubio CA, Mini E, Nesi G. Genomic and genetic alterations influence the progression of gastric cancer. *World J Gastroen-*

terol 2011; **17**: 290-299
24 **Pandey R**, Misra V, Misra SP, Dwivedi M, Kumar A, Tiwari BK. Helicobacter pylori and gastric cancer. *Asian Pac J Cancer Prev* 2010; **11**: 583-588
25 **Hatziapostolou M**, Iliopoulos D. Epigenetic aberrations during oncogenesis. *Cell Mol Life Sci* 2011; **68**: 1681-1702
26 **Rodríguez-Paredes M**, Esteller M. Cancer epigenetics reaches mainstream oncology. *Nat Med* 2011; **17**: 330-339
27 **Watanabe Y**, Maekawa M. Methylation of DNA in cancer. *Adv Clin Chem* 2010; **52**: 145-167
28 **Corvalan AH**, Maturana MJ. Recent patents of DNA methylation biomarkers in gastrointestinal oncology. *Recent Pat DNA Gene Seq* 2010; **4**: 202-209
29 **Knight RF**, Bader DM, Backstrom JR. Membrane topology of Bves/Pop1A, a cell adhesion molecule that displays dynamic changes in cellular distribution during development. *J Biol Chem* 2003; **278**: 32872-32879
30 **Ripley AN**, Osler ME, Wright CV, Bader D. Xbves is a regulator of epithelial movement during early Xenopus laevis development. *Proc Natl Acad Sci USA* 2006; **103**: 614-619
31 **Ripley AN**, Chang MS, Bader DM. Bves is expressed in the epithelial components of the retina, lens, and cornea. *Invest Ophthalmol Vis Sci* 2004; **45**: 2475-2483
32 **Osler ME**, Chang MS, Bader DM. Bves modulates epithelial integrity through an interaction at the tight junction. *J Cell Sci* 2005; **118**: 4667-4678
33 **Hager HA**, Roberts RJ, Cross EE, Proux-Gillardeaux V, Bader DM. Identification of a novel Bves function: regulation of vesicular transport. *EMBO J* 2010; **29**: 532-545
34 **Gingold-Belfer R**, Bergman M, Alcalay Y, Schlesinger H, Aravot D, Berman M, Salman H, Brand T, Kessler-Icekson G. Popeye domain-containing 1 is down-regulated in failing human hearts. *Int J Mol Med* 2011; **27**: 25-31
35 **Jayagopal A**, Yang JL, Haselton FR, Chang MS. Tight junction-associated signaling pathways modulate cell proliferation in uveal melanoma. *Invest Ophthalmol Vis Sci* 2011; **52**: 588-593
36 **Feng Q**, Hawes SE, Stern JE, Wiens L, Lu H, Dong ZM, Jordan CD, Kiviat NB, Vesselle H. DNA methylation in tumor and matched normal tissues from non-small cell lung cancer patients. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 645-654
37 **Wells A**, Yates C, Shepard CR. E-cadherin as an indicator of mesenchymal to epithelial reverting transitions during the metastatic seeding of disseminated carcinomas. *Clin Exp Metastasis* 2008; **25**: 621-628
38 **Yilmaz M**, Christofori G. EMT, the cytoskeleton, and cancer cell invasion. *Cancer Metastasis Rev* 2009; **28**: 15-33
39 **Micalizzi DS**, Farabaugh SM, Ford HL. Epithelial-mesenchymal transition in cancer: parallels between normal development and tumor progression. *J Mammary Gland Biol Neoplasia* 2010; **15**: 117-134
40 **Williams CS**, Zhang B, Smith JJ, Jayagopal A, Barrett CW, Pino C, Russ P, Presley SH, Peng D, Rosenblatt DO, Haselton FR, Yang JL, Washington MK, Chen X, Eschrich S, Yeatman TJ, El-Rifai W, Beauchamp RD, Chang MS. BVES regulates EMT in human corneal and colon cancer cells and is silenced via promoter methylation in human colorectal carcinoma. *J Clin Invest* 2011; **121**: 4056-4069

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CASE REPORT

Giardia infection: Protein-losing enteropathy in an adult with immunodeficiency

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Abstract

The case of a 52-year-old woman with a past history of thymoma resection who presented with chronic diarrhea and generalized edema is the focal point of this article. A diagnosis of *Giardia lamblia* infection was established, which was complicated by protein-losing enteropathy and severely low serum protein level in a patient with no urinary protein loss and normal liver function. After anti-helmintic treatment, there was recovery from hypoalbuminemia, though immunoglobulins persisted at low serum levels leading to the hypothesis of an immune system disorder. Good's syndrome is a rare cause of immunodeficiency characterized by the association of hypogammaglobulinemia and thymoma. This primary immune disorder may be complicated by severe infectious diarrhea secondary to disabled humoral and cellular immune response. This is the first description in the literature of an adult patient with an immunodeficiency syndrome who presented with protein-losing enteropathy secondary to giardiasis.

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Key words: Chronic diarrhea; Giardiasis; Protein-losing enteropathy; Immunodeficiency

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INTRODUCTION

Protein-losing enteropathy (PLE) is a rare syndrome of gastrointestinal protein loss that may complicate a variety of diseases. The diagnosis of PLE should be considered in patients with hypoproteinemia, especially hypoalbuminemia, after other causes have been excluded, such as malnutrition, proteinuria, and impaired protein synthesis. The diagnosis is most commonly based on the determination of fecal α-1 antitrypsin clearance, though scintigraphy (technetium-99m-labeled human serum albumin scan-^{99m}Tc-HSA) aids in localizing the site and quantifying enteric protein loss[1].

The pathogenic mechanism can be divided into erosive gastrointestinal disorders, nonerosive gastrointestinal disorders, and disorders involving increased central venous pressure or mesenteric lymphatic obstruction[1]. Al-

though not extensively studied, certain enteric infections (*Salmonella*, *Shigella*, *Rotavirus*, and *Giardia lamblia*) can also damage the intestinal mucosa leading to excessive protein loss, particularly in an immunocompromised host[2,3].

Giardia lamblia is a protozoan that frequently infects the gut and plays an important role in the public health of developing countries, such as Brazil[4]. Transmission occurs through oral-fecal contact or intake of contaminated water and food by the infecting forms (cysts or oocysts). Although normally presenting with mild symptoms, such as cramps and chronic diarrhea, more severe complications can take place and malabsorptive syndrome may develop[5]. In this case report, severe giardiasis is illustrated in an adult patient with a past history of thymoma who developed PLE, a rare complication that particularly affects infants.

CASE REPORT

A 52-year-old black woman was referred to our hospital because of chronic diarrhea and generalized edema. She had gained 10 kg over the last year because of peripheral edema, and complained about asthenia and fatigue. She had no abdominal pain, fever or stool bleeding. Two years before the event, while under investigation for chronic cough and chest pain, the diagnosis of a type AB thymoma was made according to World Health Organization classification and she underwent a sternal thymectomy. Anatomopathological analysis showed no signs of capsular, vascular or lymphatic invasion.

The patient presented mild steatorrhea on a semi-quantitative stool analysis, negative proctoparasitologic examination and severe hypoproteinemia on serum protein electrophoresis (Figure 1A). A hepatic cause of hypoproteinemia was ruled out by clinical and laboratory parameters and urinary sedimentary analysis was normal with no proteinuria. There was altered coagulation testing related to vitamin K deficiency, normocytic normochromic anemia, and normal leucocyte and platelet count.

She was submitted to ^{99m}Tc-HSA which demonstrated mild protein loss from the small bowel though no specific site was able to be localized. She had a normal colonoscopy and an upper digestive endoscopy that showed moderate duodenitis. On duodenal biopsy, there were structures identified as *Giardia lamblia,* moderate non-specific active chronic duodenitis, and preserved villi:crypt ratio. Giardiasis was not attributed at first to be the cause of the enteric protein loss. In subsequent investigations, anterograde enteroscopy was undergone, demonstrating only non-specific duodenal and jejunal lesions. No remarkable findings were verified on histological samples.

Nevertheless, after anti-helmintic treatment with oral metronidazole, she ended up recovering from diarrhea and edema. No signs of enteric protein loss were verified by scintigraphy and serum albumin level normalized, though hypogammaglobulinemia persisted (Figure 1B).

On evaluation of intermittent asthma and rhinitis since childhood and recurrent pneumonia, antibody deficiency was diagnosed, including immunoglobulins IgA, IgE, IgG and IgM. Cytometry analysis of peripheral blood lymphocytes revealed a marked decrease in CD19$^+$ B-cells. Bone marrow biopsy demonstrated a chromosome 9 inversion with chromosome 16 deletion [*46,X X,inv(9),del(16)(q22)*] with unknown significance and no additional important findings. Based on previous history of thymoma and current hypogammaglobulinemia, the diagnosis of Good's syndrome (GS) was made even though no evidence of cell-mediated immune defects was verified.

DISCUSSION

Giardia lamblia is a flagellated intestinal protozoan with oral-fecal transmission. Infection takes place in the proximal small gut, especially duodenum, where motile trophozoites live adhered to enterocytes[5].

Examination of concentrated, iodine-stained wet stool preparations and modified-trichrome-stained permanent smears has been the conventional approach to the diagnosis. Despite three negative stool samples, the diagnosis of giardiasis could be made by duodenal biopsy in this case report. In fact, cysts and trophozoites are present only intermittently in feces, offering a low sensitivity of approximately 50%, even with examination of multiple specimens. Identification of trophozoites within small intestinal biopsy specimens requires careful examination of multiple microscope fields to ensure accuracy[6], although on direct sampling of duodenal contents (e.g., duodenal aspiration or the "string test"), sensitivity can be improved to approximately 80%. Therefore molecular tests based on enzyme linked immunosorbent assay or direct immunofluorescent antibody microscopy should be the first diagnostic test to be performed because of high accuracy, with sensitivities greater than 90% and specificities approaching 100%.

Worldwide, *Giardia* affects infants more commonly than adults and has different pathogenicity in experimental human infections. Infected patients, especially those immunocompromised, may present malabsorptive diarrhea of yet unknown mechanism. Trophozoites adhere (perhaps by suction) to the epithelium of the upper small intestine, using a disk structure located on their ventral surface. There is no evidence that trophozoites invade the mucosa. On biopsy, pathologic changes range from an entirely normal-appearing duodenal mucosa (except for adherent trophozoites) to severe villous atrophy with a mononuclear cell infiltrate that resembles celiac sprue[7].

The severity of diarrhea appears to correlate with the severity of the pathologic change[6]. In fact, the host immune response plays a critical role in limiting the severity of giardiasis; this is mediated by humoral immune response, both systemic (IgM and IgG) and mucosal (IgA). Certain populations, including children younger than 2 years of age and patients with hypogammaglobulinemia, are more likely to develop serious disease. When infected with *Giardia*, individuals with common variable immuno-

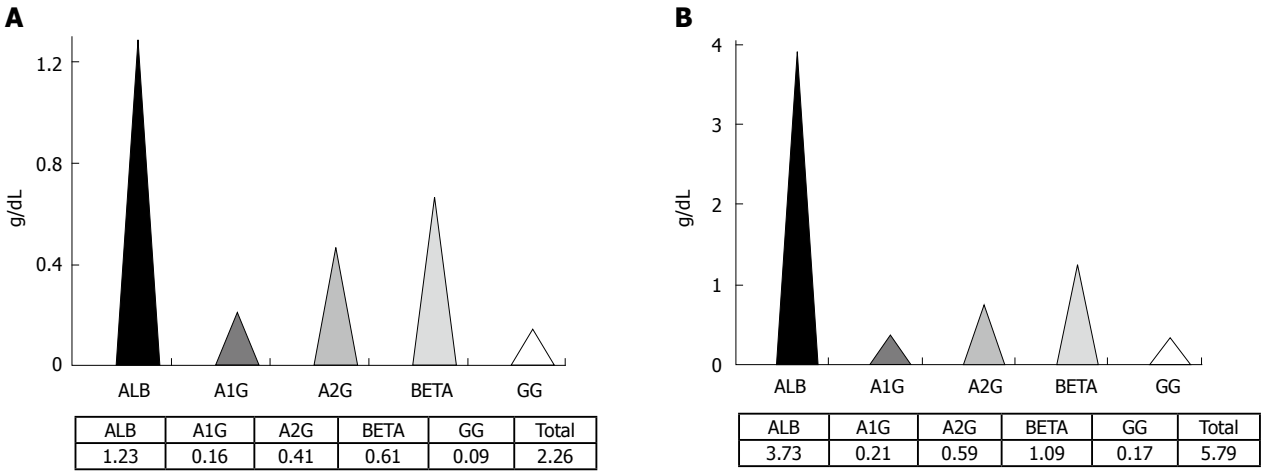

Figure 1 Serum protein electrophoresis analysis before treatment (A) and after treatment (B). ALB: Albumin; A1G: α-1 globulin; A2G: α-2 globulin; BETA: β globulin; GG: γ globulin.

deficiency develop severe, protracted diarrhea and malabsorption with sprue-like pathologic changes that resolve with treatment[5] such as immunoglobulin replacement[6]. The importance of a cellular immune response is yet to be determined. On immune reconstitution, severe inflammatory changes and villous atrophy develop in the intestine, suggesting that the immune response to infection may contribute to pathology, although severe infections that are resistant to treatment or an increased frequency of giardiasis have been noted only rarely in patients with acquired immune deficiency syndrome[7].

PLE is a rare complication associated with Giardia infection, as formerly described in infants[1-3]. As illustrated in this article, this infection may cause severe enteric mucosal damage that leads to an inflammatory process and exudative protein loss. Sometimes, especially when there is associated lymph leakage, secondary immunodeficiency may develop as decreases in serum lymphocytes and hypogammaglobulinemia occur[1].

As previously mentioned, although PLE is better diagnosed by the determination of fecal α-1 antitrypsin clearance because of high accuracy of the method, scintigraphy was the option chosen for the diagnosis in this case and it could localize protein loss from the small gut at non-specific sites. ^{99m}Tc-HSA scan possesses high sensitivity and specificity; it may demonstrate the site and semi-quantify protein loss, despite some false positives resulting from active gastrointestinal bleeding and *in vivo* breakdown of ^{99m}Tc-HSA, yielding free pertechnetate from radiolabeling[8]. The former is easily excluded by fecal occult blood examination and the latter by the absence of stomach or thyroid visualization.

At first, the hypothesis of hypogammaglobulinemia secondary to gut protein loss was put forward for this patient since severe universal hypoproteinemia was verified in pre-treatment serum protein electrophoresis. Nevertheless, after metronidazole was instituted, every serum protein fraction increased but gammaglobulin remained very low. This led to the belief that she possessed an immune disorder, which could explain the advanced enteropathy caused by *Giardia* infection and the persistent hypogammaglobulinemia in spite of medical treatment.

Patients with thymoma may suffer from specific types of paraneoplastic syndrome or remote effects of cancer which have underlying autoimmunity origins. They include myasthenia gravis, pure red cell aplasia, and hypogammaglobulinemia (Good's syndrome), with approximate occurrence of 30%, from 1.6% to 5%, and from 3% to 6%, respectively. Autoimmunity to B lymphocyte lineage causes severe deficiency in B lymphocytes and hypogammaglobulinemia, resulting in acquired immunodeficiency vulnerable especially to bacterial infection. Although most paraneoplastic syndromes have been documented to recover after tumor resection, some previous case reports seem to indicate that hypogammaglobulinemia without improvement can last even after tumor resection for as long as 9 years[9].

GS is a rare association of thymoma and immunodeficiency first described more than 50 years ago by Dr. Good. The diagnosis of thymoma usually precedes the diagnosis of hypogammaglobulinemia and other clinical manifestations, such as infection or diarrhea, in almost half of the cases. Recurrent sinopulmonary infection is commonly the first clinical manifestation followed by chronic diarrhea, the mechanism of which is not identified in the majority of cases, although bacteria, such as *Salmonella spp.*, and other pathogens, like cytomegalovirus and *Giardia lamblia* might the etiological factor[9,10].

GS, in contrast to other primary immunodeficiency disorders such as common variable immunodeficiency disease, usually affects adults in the fourth or fifth decade of life, equally male and female, and almost always associated with hypogammaglobulinemia. Although there are no formal diagnostic criteria for this disorder, GS is characterized by low to absent B cells in the peripheral blood, hypogammaglobulinemia, and variable defects in cell-mediated immunity; with a CD4 lymphopenia, an inverted CD4/CD8+ T-cell ratio and reduced T-cell mitogen pro-

liferative responses[4]. In clinical practice, as illustrated in this report, serum flow lymphocytometry shows absence of B lymphocytes, discrete low CD4 T lymphocyte count and inverted CD4/CD8 ratio[9-11].

It has long been known that giardiasis is a frequent infection of the gastrointestinal tract, especially in infants and in developing countries, typically manifesting as chronic diarrhea, abdominal cramps and fatigue. The diagnosis may be challenging as conventional methods of stool examination can lack sensitivity. In cases of strong clinical suspicion, serum serology is of great value when available, though upper digestive endoscopy is a reliable tool to demonstrate degree of duodenal damage and direct diagnosis can be made through histological sample. As far as we know, this is the first time in the literature that Giardia infection complicated with PLE has been reported in an adult patient. In fact, whenever severe giardiasis occurs in adulthood, immunodeficiency should always be kept in mind because of humoral IgA-mediated immunological pathogenicity, and clinical investigation should be directed towards this suspicion, even though rare in cause.

REFERENCES

1 **Umar SB**, DiBaise JK. Protein-losing enteropathy: case illustrations and clinical review. *Am J Gastroenterol* 2010; **105**: 43-49; quiz 50

2 **Braamskamp MJ**, Dolman KM, Tabbers MM. Clinical practice. Protein-losing enteropathy in children. *Eur J Pediatr* 2010; **169**: 1179-1185

3 **Dubey R**, Bavdekar SB, Muranjan M, Joshi A, Narayanan TS. Intestinal giardiasis: an unusual cause for hypoproteinemia. *Indian J Gastroenterol* 2000; **19**: 38-39

4 **Mascarini LM**, Donalísio MR. [Giardiasis and cryptosporidiosis in children institutionalized at daycare centers in the state of São Paulo]. *Rev Soc Bras Med Trop* 2006; **39**: 577-579

5 **Huston CD.** Chapter 109: Intestinal protozoa-Giardia lamblia. In: Feldman M, Friedman LS, Brandt LJ, editors. Sleisenger and Fordtran's gastrointestinal and liver disease: pathophysiology/diagnosis/management. 8th ed. Philadelphia: Elsevier Inc., 2006: 1911-1914

6 **Roxström-Lindquist K**, Palm D, Reiner D, Ringqvist E, Svärd SG. Giardia immunity--an update. *Trends Parasitol* 2006; **22**: 26-31

7 **Solaymani-Mohammadi S**, Singer SM. Giardia duodenalis: the double-edged sword of immune responses in giardiasis. *Exp Parasitol* 2010; **126**: 292-297

8 **Chen YC**, Hwang SJ, Chiu JS, Chuang MH, Chung MI, Wang YF. Chronic edema from protein-losing enteropathy: scintigraphic diagnosis. *Kidney Int* 2009; **75**: 1124

9 **Kelleher P**, Misbah SA. What is Good's syndrome? Immunological abnormalities in patients with thymoma. *J Clin Pathol* 2003; **56**: 12-16

10 **Kelesidis T**, Yang O. Good's syndrome remains a mystery after 55 years: A systematic review of the scientific evidence. *Clin Immunol* 2010; **135**: 347-363

11 **Kitamura A**, Takiguchi Y, Tochigi N, Watanabe S, Sakao S, Kurosu K, Tanabe N, Tatsumi K. Durable hypogammaglobulinemia associated with thymoma (Good syndrome). *Intern Med* 2009; **48**: 1749-1752

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CASE REPORT

An unusual enteropathy-associated T-cell lymphoma with MYC translocation arising in a Japanese patient: A case report

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Abstract

Enteropathy-associated T-cell lymphoma (EATL) is a rare peripheral T-cell lymphoma classified into 2 types, with or without celiac disease, based on histology. Type 2 EATL is less commonly associated with celiac disease, in which cells are characterized by being monomorphic and small- to medium-sized. Cells are characterized by CD8 and CD56 expression and c-MYC oncogene locus gain. We present an atypical case of type 2 EATL in the jejunum, with human T-lymphotropic virus-1 that was CD4- CD8+ CD56- CD30- CD25- TIA-1+ and granzyme B+ on immunohistological staining. It also displayed translocation of chromosome 8p24 (c-MYC), as determined by fluorescent *in situ* hybridization. Mucosal spreading and intraepithelial invasion by lymphoma with villous atrophy were detected adjacent to the mucosal layer. The lymphoma may be derived from intraepithelial CD8+ T cells, similar to celiac disease.

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Key words: Enteropathy-associated T-cell lymphoma; Celiac disease; Human T-lymphotropic virus-1; Fluorescent in situ hybridization; Chromosome 8p24

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INTRODUCTION

Gastrointestinal lymphomas account for 4%-20% of all non-Hodgkin's lymphomas. Enteropathy-associated T-cell lymphoma (EATL) is a primary extranodal T-cell lymphoma arising in the gastrointestinal tract, a rare subtype of peripheral T-cell lymphoma, and accounts for less than 1% of all non-Hodgkin's lymphomas[1-3]. There are two different sub-classifications: type 1 EATL (with) and type 2 EATL (without) celiac disease[1,4]. The disease is seen with greater frequency in areas with a high prevalence of celiac disease, but it is rare in Japan[5]. Type 2 EATL accounts for 10%-20% of cases, and is less commonly associated

with celiac disease[1,4]. The tumor cells are monomorphic and small- to medium-sized, with infiltration of the intestinal crypt epithelium without inflammation[1,4]. Type 2 EATL often occurs without a history of celiac disease and shows strong expression of CD56 (> 90%) and CD8 (80%) based on immunohistochemical staining[1]. EATL generally has a poor prognosis because it is often diagnosed late, has spread, and is therefore refractory to treatment. We report a case with human T-lymphotropic virus-1 (HTLV-1) in which a type 2 EATL was CD8+, CD56-, T cell restricted intracellular antigen-1 (TIA-1)+ on immunohistological staining, and possessed a unique chromosomal change as determined by fluorescent *in situ* hybridization (FISH).

CASE REPORT

A 66-year-old woman with a past medical history of hypertension presented to the emergency department with a sudden onset of severe abdominal pain. Her weight was 43.5 kg and body mass was 19.7 kg/m^2. A physical examination revealed a high fever (39 ℃) with chills; diffuse, severe, constant abdominal pain; and muscular guarding. She had experienced abdominal distension for a month, but did not have diarrhea, weight loss, a history of malnutrition, or a food intolerance. We could not detect any palpable peripheral lymph nodes. The laboratory data on admission showed mild leukocytosis (white blood cell count of 10 400/mm^3) and hypoproteinemia (total protein 5.4 g/dL). Persistent hypoproteinemia was recognized at 4 mo prior, based on previous medical records. No atypical lymphocytes were detected in the peripheral blood. A computed tomography showed free air and wall thickening in the pseudo-aneurysmally dilated small bowel within the pelvic area, with a defect of the intestinal wall (Figure 1). Laparotomy was performed and showed a pseudo-aneurysmally dilated and perforated jejunum 80 cm from the ligament of Treitz, with massive purulent ascites fluid. We performed a segmental resection of the lesion and a side-to-side anastomosis. The resected jejunum was generally thickened and there was diffuse transmural infiltration by monomorphic and medium-sized atypical lymphoid cells, with villous atrophy of the intestinal glands (Figure 2A). Intramucosal spreading of lymphoma was found in an adjacent mucosal layer, and many atypical intraepithelial lymphocytes were detected (Figure 2B and C). Immunohistochemical staining was positive for CD3, CD7, CD8, TIA-1 granzyme B, c-MYC and Ki-67, and negative for CD4, CD5, CD20, CD25, CD30, CD56, and CCR4 (Figure 2D and E). FISH analysis revealed translocation of chromosome 8q24 (c-MYC) (Figure 2F). Polymerase chain reaction analysis of *TCRγ* gene rearrangements were performed using the BIOMED-2 procedure[5]. Rearrangement bands were detected in 4 VγIf, Vγ9, Vγ10, Vγ11, and Jγ1.1/2.1, and Jγ1.3/2.3 consensus primers.

Based on these findings, we made a diagnosis of type 2 EATL. On postoperative day 10, the patient was discharged without any complications. After the diagnosis, we performed additional laboratory tests. Serum soluble interleukin-2 receptor was elevated (822 U/mL), and serum HTLV-1 antibodies were positive. 67 Gallium scintigraphy and fluorodeoxyglucose positron-emission tomography did not show any site of involvement. Bone marrow biopsy did not reveal malignant cells. Based on these results, we diagnosed clinical stage 2E. The patient received high-dose chemotherapy with autologous hematopoietic cells 6 mo after surgery.

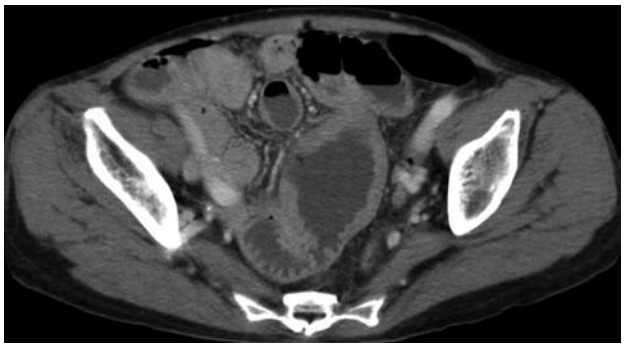

Figure 1 Computed tomography scan of the dilated and perforated small bowel.

DISCUSSION

EATL is a rare subtype of peripheral T-cell lymphoma, with an incidence of 0.25% of all lymphomas in Japan[3,6]. Since gluten allergy is uncommon in Japan, it is extremely rare for celiac disease to be a basal disorder, and type 2 EATL is more common than type 1 EATL[5]. EATL most commonly arises in the proximal jejunum, and typically presents with abdominal pain. It is often associated with intestinal obstruction, perforation, or bleeding, and is diagnosed by histology tests. Type 2 EATL consists of monomorphic and small- to medium-sized tumor cells that frequently express CD8 and CD56[3]. The prognosis of EATL is usually poor based on results of conventional combination chemotherapy, although high-dose chemotherapy with autologous hematopoietic cell transplantation may have longer survival rates[2,7].

The pathological findings of this case showed monomorphic, small- to medium-sized tumor cells with villous atrophy of the mucosa. Combined with the mucosal spreading and increased intraepithelial lymphocytes (IELs), we made a diagnosis of type 2 EATL. Type 2 EATL is usually positive for CD8 and CD56 (> 90%), but this patient was CD56 negative[3]. Several studies have suggested that the Epstein-Barr virus plays an etiological role in EATL pathogenesis, but this is still a subject of debate. In this case, Epstein-Barr encoded RNAs and latent membrane proteins were not expressed, suggesting that Epstein-Barr virus was not involved in tumor proliferation. Test results showed HTLV-1 infection based on serological antibodies, but the tumor cells were different from adult T cell lymphoma/leukemia immunopheno-

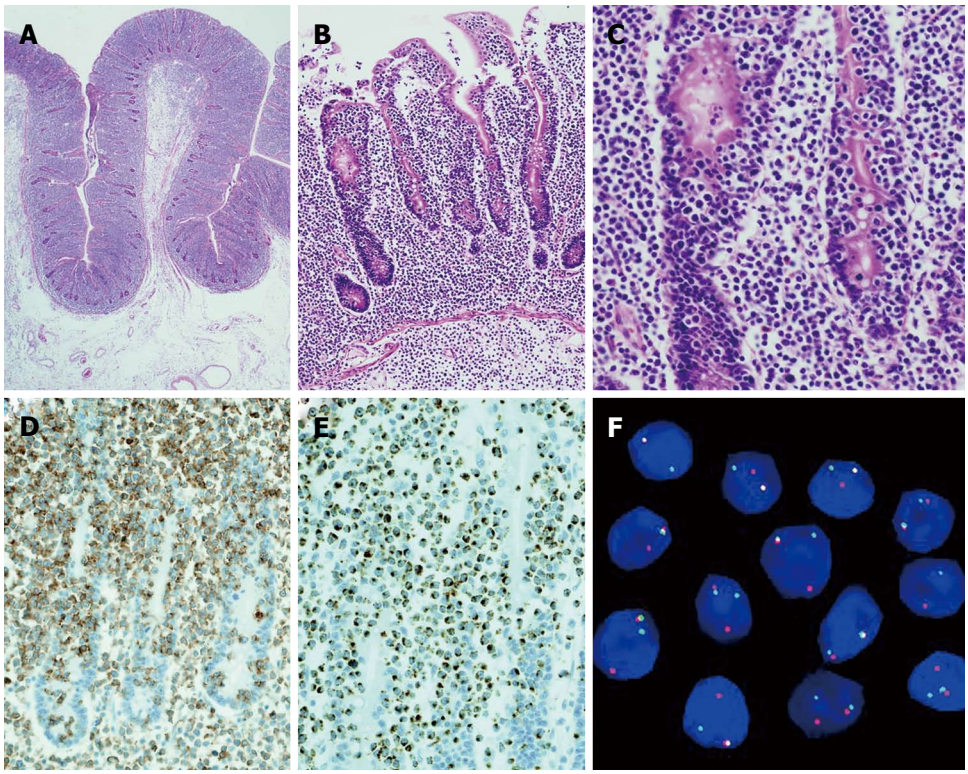

Figure 2 Pathological findings of the specimen. A: Microscopic findings of the specimen with villous atrophy, crypt hyperplasia, and proliferation of intraepithelial lymphocytes; B: Mucosal and submucosal invasion by lymphoma cells; C: Lymphoma cells with the characteristics of intraepithelial lymphocytes; D: Expression of CD8 by intraepithelial lymphocytes; E: Expression of T cell restricted intracellular antigen-1 by intraepithelial lymphocytes; F: Examination of chromosome 8q24 (c-MYC region) using break point rearrangement probe by *in situ* hybridization.

type features with respect to CD4, CD8, CD25, CCR4 and cytotoxic proteins[8]. FISH analysis, however, showed gene translocation at 8q24, which is the c-MYC oncogene locus. It has been suggested that c-MYC protein plays a role in lymphoid proliferation and the apoptotic pathway in malignant lymphomas[9]. In type 2 EATL pathogenesis, about 70% of cases show a gain of 8q24, suggesting c-MYC is an important transcription factor in EATL[4]. We present here the first EATL case with translocation of chromosome 8q24 (c-MYC region).

Some studies suggested chronic inflammation might contribute to the development of neoplastic lymphocyte growth in EATL[4,10]. Celiac disease is characterized by villous atrophy, crypt hyperplasia, and an increased invasion of CD8+ IEL. Celiac disease may progress to type 1 EATL[10], especially in patients with refractory celiac disease, in which IELs have aberrant phenotypes and genetic alterations due to persistent inflammation[4,10]. This is supported by the fact that refractory celiac disease and type 1 EATL rarely show aberrant CD8 expression[4,10]. This case showed protein-losing enteropathy and lymphoma cells with IEL-like features in the mucosa, and villous atrophy. We considered the possibility of persistent enteropathy, which is consistent with hypoproteinemia 4 mo before the surgery. Celiac-like enteropathy, rare in Japan, is consistent with the clinical symptoms, and in this case, persistent enteropathy might cause CD8+ IELs to transform to neoplastic cells *via* c-MYC. The immunophenotype of this case was different from typical type 2 EATL cases, regarding CD3+ CD4- CD8+ CD30- CD56-.

In summary, we report the unique case of a 66-year-old HTLV-1 woman diagnosed with type 2 EATL, which was CD3+ CD4- CD8+ CD30- CD56- on immunostaining, and displayed translocation of the c-MYC region.

REFERENCES

1 **Swerdlow SH,** Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC Press, 2008: 439

2 **Gale J**, Simmonds PD, Mead GM, Sweetenham JW, Wright DH. Enteropathy-type intestinal T-cell lymphoma: clinical features and treatment of 31 patients in a single center. *J Clin Oncol* 2000; **18**: 795-803

3 **Zettl A**, deLeeuw R, Haralambieva E, Mueller-Hermelink HK. Enteropathy-type T-cell lymphoma. *Am J Clin Pathol* 2007; **127**: 701-706

4 **Deleeuw RJ**, Zettl A, Klinker E, Haralambieva E, Trottier M, Chari R, Ge Y, Gascoyne RD, Chott A, Müller-Hermelink HK, Lam WL. Whole-genome analysis and HLA genotyping of enteropathy-type T-cell lymphoma reveals 2 distinct lymphoma subtypes. *Gastroenterology* 2007; **132**: 1902-1911

5 **Takeshita M,** Nakamura S, Kikuma K, Nakayama Y, Nimura S, Yao T, Urabe S, Ogawara S, Yonemasu H, Matsushita Y, Karube K, Iwashita A. Pathological and immunohistological findings and genetic aberrations of intestinal enteropathy-associated T cell lymphoma in Japan. *Histopathology* 2011; **58**: 395-407

6 **Nakamura S**, Matsumoto T, Iida M, Yao T, Tsuneyoshi M.

Primary gastrointestinal lymphoma in Japan: a clinicopathologic analysis of 455 patients with special reference to its time trends. *Cancer* 2003; **97**: 2462-2473

7 **Sieniawski M**, Angamuthu N, Boyd K, Chasty R, Davies J, Forsyth P, Jack F, Lyons S, Mounter P, Revell P, Proctor SJ, Lennard AL. Evaluation of enteropathy-associated T-cell lymphoma comparing standard therapies with a novel regimen including autologous stem cell transplantation. *Blood* 2010; **115**: 3664-3670

8 **Tsukasaki K**, Hermine O, Bazarbachi A, Ratner L, Ramos JC, Harrington W, O'Mahony D, Janik JE, Bittencourt AL, Taylor GP, Yamaguchi K, Utsunomiya A, Tobinai K, Watanabe T. Definition, prognostic factors, treatment, and response criteria of adult T-cell leukemia-lymphoma: a proposal from an international consensus meeting. *J Clin Oncol* 2009; **27**: 453-459

9 **Knudsen A**. The influence of the reserve albumin concentration and pH on the cephalocaudal progression of jaundice in newborns. *Early Hum Dev* 1991; **25**: 37-41

10 **de Mascarel A**, Belleannée G, Stanislas S, Merlio C, Parrens M, Laharie D, Dubus P, Merlio JP. Mucosal intraepithelial T-lymphocytes in refractory celiac disease: a neoplastic population with a variable CD8 phenotype. *Am J Surg Pathol* 2008; **32**: 744-751

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CASE REPORT

Spontaneous perforation of an intramural rectal hematoma: Report of a case

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Abstract

Spontaneous hematomas are rare and most occur secondary to hematologic disorders or during anticoagulant therapy. Most spontaneous hematomas occur above the sigmoid colon, and rarely in the rectum. Herein we present the case of a patient with a spontaneous perforating hematoma of the rectum who presented with severe abdominal pain after a bloody stool. The hemoglobin level decreased by 33 g/L within 20 h. An abdominal sonogram showed a hydrops in the lower abdomen with a maximum depth of 7.0 cm. A hematoma, 8 cm × 6 cm × 5 cm in size, was noted intra-operatively in the rectosigmoid junction, with a 1.5-cm perforation in the hematoma and active hemorrhage. Thus, a partial rectectomy and sigmoidostomy were performed. Three months later, a second operative procedure to re-establish intestinal continuity was performed. The patient is in good condition 12 mo after the last surgery. In addition to this case, the causes of spontaneous perforating hematomas and the treatment are discussed.

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Key words: Rectum; Hematoma; Complications; Prothrombin time; Diagnosis; Therapy

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INTRODUCTION

Intramural hematomas may occur in every portion of the alimentary tract from the esophagus to the sigmoid colon, but rarely occur in the rectum. Intramural hematomas most often result from blunt trauma[1]. Non-traumatic spontaneous hematomas usually reflect an underlying blood dyscrasia, anticoagulant therapy, and hematologic diseases[2]. Spontaneous rectal hematomas have rarely been reported. Herein we present this unusual case of a spontaneous perforating hematoma.

CASE REPORT

A 52-year-old male was admitted to our hospital with a 20-h history of severe abdominal pain after passing a small amount of bloody stool. The patient had no history of trauma before admission and clearly related the acute onset of pain with straining to have a bowel movement. The patient had 5-year history of oral warfarin sodium use after mitral valve replacement and coronary stent implantation. The physical examination revealed diffuse abdominal tenderness, rebound tenderness, widespread perianal ecchymosis, and muscular tension without na-

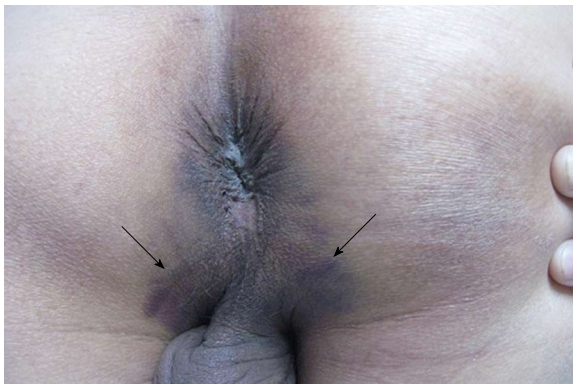

Figure 1 Pre-operative photograph of the perineum (knee-chest position) revealing widespread perianal ecchymosis (marked with black arrows).

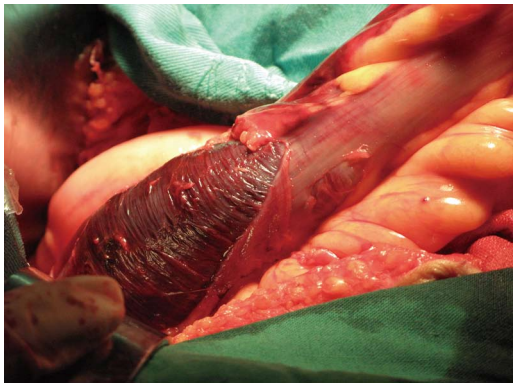

Figure 2 Intra-operative photograph of a large intramural hematoma arising beneath the rectosigmoid junction.

usea and vomiting (Figure 1). There was a persistent drop in hemoglobin from 94 g/L to 61 g/L over a 20-h interval. The prothrombin time was prolonged (44.40 s; approximately four times the normal prothrombin time). An abdominal sonogram showed a large hydrops in the lower abdomen with a maximum depth of 7.0 cm. After admission, active resistance to shock treatment was given, and the abdominal symptoms did not remit, thus emergent surgery was performed. A palpable hematoma, 8 cm × 6 cm × 5 cm in size, was noted in the rectosigmoid junction intra-operatively (Figure 2). Furthermore, there was a perforation in the hematoma, 1.5 cm in diameter, and active hemorrhage. After confirming that the perforating hematoma of the rectum was the cause of the generalized peritonitis and the extensive oozing of blood could not be stopped, a partial rectectomy and sigmoidostomy were performed. A histopathologic examination of the removed specimen revealed extensive oozing of blood in the muscular layer of the rectum and fibrinoid necrosis within the vascular wall (Figure 3). The patient recovered without complications and was discharged on the 8th post-operative day. Three months later, the patient returned for re-establishment of intestinal continuity. The patient was in good health and completely asymptomatic during the 12-mo follow-up period after the second surgical procedure.

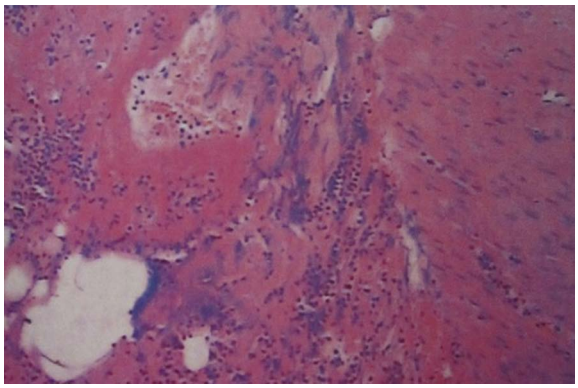

Figure 3 Extensive oozing of blood in the muscular layer of the resected rectal and fibrinoid necrosis within the vascular wall (hematoxylin and eosion stain, × 20).

DISCUSSION

Intramural hematomas of the alimentary tract are unusual. Intramural hematomas mainly occur above the sigmoid colon, and rarely in the rectum. The duodenum is the most common site for intramural hematomas[1]. In a review of the medical literature, most rectal intramural hematomas occur following blunt trauma. Spontaneous perforating hematomas of the rectum have rarely been reported[3].

Of all intramural hematomas, 15%-36% are spontaneous in patients with underlying hematologic diseases or on anticoagulant therapy[2]. As shown in the current case, the patient had a long history of oral warfarin sodium use, and the prothrombin time was prolonged to 44.40 s, which is approximately four times the normal prothrombin time. Anticoagulation-induced intramural hematomas can occur in patients with therapeutic prothrombin times, but the majority of intramural hematomas occur in patients with abnormally prolonged prothrombin times[2]. Thus, anticoagulant therapy was thought to be closely related with the hematoma in this patient. In addition, increased abdominal pressure during straining may play an important role in hematoma formation. Specifically, when a patient strains to have a bowel movement, the abdominal pressure increases and the rectum contracts. The contracted rectum increases the intramural pressure which leads to decreased vascular compliance, and even rupture, thus causing a rectal intramural hematoma. When the pressure of the intramural hematoma is greater than the capacity of the hematoma wall, the hematoma breaks and clinical symptoms appear.

Most patients with intramural hematomas present with signs of intestinal obstruction[3]. Conservative therapy is usually sufficient because the hematomas will undergo spontaneous resorption[4], but treatment decisions depend on the symptoms and clinical findings[4,5]. Relief of the tamponade effect during conservative therapy could lead to further bleeding[1]. In patients in whom the cause of obstruction is not known, or in patients with complete obstruction or who have failed medical management, surgical intervention is required[6]. Draining the

hematoma might increase the risk of serious infection, as reported previously[7]. According to the decreasing hemoglobin concentration and failed conservative therapy, we performed a partial rectectomy and sigmoidostomy. The patient recovered without complications.

Therefore, testing prothrombin times regularly and avoiding severe abdominal pressure in a patient who has a long-term oral anticoagulant drug history might help prevent complications associated with intramural hematomas, such as perforation.

REFERENCES

1 **McClenathan JH**, Dabadghav N. Blunt rectal trauma causing intramural rectal hematoma: report of a case. *Dis Colon Rectum* 2004; **47**: 380-382

2 **Hughes CE**, Conn J, Sherman JO. Intramural hematoma of the gastrointestinal tract. *Am J Surg* 1977; **133**: 276-279

3 **TerKonda SP**, Nichols FC, Sarr MG. Spontaneous perforating hematoma of the rectum. Report of a case. *Dis Colon Rectum* 1992; **35**: 270-272

4 **Zangan SM**, Yousefzedah DK. Occlusive intraluminal hematoma. *Pediatr Radiol* 2004; **34**: 564-566

5 **Babu ED**, Axisa B, Taghizadeh AK, Delicata RJ. Acute spontaneous haematoma of the rectum. *Int J Clin Pract* 2001; **55**: 66-67

6 **Battal B**, Kocaoglu M, Ors F, Akgun V, Tasar M. Obstructive rectal intramural hematoma caused by a foreign body. *Emerg Radiol* 2009; **16**: 75-77

7 **Chen YM**, Davis M, Ott DJ. Traumatic rectal hematoma following anal rape. *Ann Emerg Med* 1986; **15**: 850-852

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LETTERS TO THE EDITOR

Does antioxidant therapy influence every aspect of quality of life?

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Abstract

To present problems that might severely impact the conclusions drawn by the authors of an article on antioxidant treatment in chronic pancreatitis (*World J Gastroenterol* 2010; 16: 4066-4071). We analyzed and discussed this paper by Shah *et al*, and found that promising as it is, this study has some methodological shortcomings, such as: cross-sectional nature of the study, lack of initial evaluations of quality of life and regular follow-ups to determine the dynamics and real directions of changes in quality of life. We therefore concluded that the results of the study by Shah *et al* are biased and, although very promising, should not be considered as scientifically relevant.

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Key words: Chronic pancreatitis; Quality of life; Methodology; Antioxidants

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TO THE EDITOR

We have read with great interest an article by Shah *et al*[1] (*World J Gastroenterol* 2010; 16: 4066-4071). Exciting news about the clinical use of antioxidant therapy seem to open an entirely new chapter in treatment of chronic pancreatitis. We have already published information on possibilities concerning the role of oxygen free radicals in experimental acute pancreatitis as well as the potential role of antioxidants in treatment the disease[2]. As a team of scientists and clinicians intensively involved in the treatment of pancreatic chronic pain, we have found the study presented by Shah *et al*[1] very promising.

Unfortunately, despite very good rationale and biochemical background, the manuscript cannot be considered as free of significant bias, that most probably severely impacts the final conclusions. The pain and quality of life (QoL) scores were obtained only after a 6-mo period of follow-up, there was no initial score at the beginning of the therapy. Moreover, the authors presented only measurement of pain and QoL, which does not seem to be sufficient in context of complexity of interactions (such as social and emotional) that chronic pancreatitis patients may experience[3,4]. In that circumstances, we would recommend that follow-up measurements should be made every month, as presented in other studies on pain in chronic pancreatitis and/or pancreatic cancer[3,4]. Lack of initial information, randomization and pain and QoL dynamics assessment leaves a suspicion that the patients treated with antioxidants suffered from less pain, had better QoL and consumed less opioids from the very beginning of this doubtlessly non-prospective, uncontrol-

led trial. Significant differences in almost every variable of QLQ-C30 seem to confirm our point of view. It is difficult to explain in any psychological paradigm or biochemical formulae, what possible influence could antioxidant therapy exert on cognitive or emotional functioning or, most interestingly on body image, alcohol-related guilt or even financial problems.

To sum up, we believe that antioxidants may offer a very interesting and promising supplementation to the treatment of chronic pancreatitis, but unfortunately, methodological problems presented in the article of Shah *et al*[1] completely disqualify the conclusions of the authors. We would be most happy to see a randomized prospective trial with dynamic evaluations that would confirm the results presented in this article.

REFERENCES

1 **Shah NS**, Makin AJ, Sheen AJ, Siriwardena AK. Quality of life assessment in patients with chronic pancreatitis receiving antioxidant therapy. *World J Gastroenterol* 2010; **16**: 4066-4071

2 **Lawinski M**, Sledzinski Z, Kubasik-Juraniec J, Spodnik JH, Wozniak M, Boguslawski W. Does resveratrol prevent free radical-induced acute pancreatitis? *Pancreas* 2005; **31**: 43-47

3 **Stefaniak T**, Vingerhoets A, Makarewicz W, Kaska L, Kobiela J, Kwiecińska B, Stanek A, Lachinski AJ, Sledziński Z. Opioid use determines success of videothoracoscopic splanchnicectomy in chronic pancreatic pain patients. *Langenbecks Arch Surg* 2008; **393**: 213-218

4 **Basinski A**, Stefaniak T, Vingerhoets A, Makarewicz W, Kaska L, Stanek A, Lachinski AJ, Sledzinski Z. Effect of NCPB and VSPL on pain and quality of life in chronic pancreatitis patients. *World J Gastroenterol* 2005; **11**: 5010-5014

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Events Calendar 2012

January 13-15, 2012
Asian Pacific *Helicobacter pylori* Meeting 2012
Kuala Lumpur, Malaysia

January 19-21, 2012
American Society of Clinical Oncology 2012 Gastrointestinal Cancers Symposium
San Francisco, CA 3000, United States

January 19-21, 2012
2012 Gastrointestinal Cancers Symposium
San Francisco, CA 94103, United States

January 20-21, 2012
American Gastroenterological Association Clinical Congress of Gastroenterology and Hepatology
Miami Beach, FL 33141, United States

February 3, 2012
The Future of Obesity Treatment
London, United Kingdom

February 16-17, 2012
4th United Kingdom Swallowing Research Group Conference
London, United Kingdom

February 23, 2012
Management of Barretts Oesophagus: Everything you need to know
Cambridge, United Kingdom

February 24-27, 2012
Canadian Digestive Diseases Week 2012
Montreal, Canada

March 1-3, 2012
International Conference on Nutrition and Growth 2012
Paris, France

March 7-10, 2012
Society of American Gastrointestinal and Endoscopic Surgeons Annual Meeting
San Diego, CA 92121, United States

March 12-14, 2012
World Congress on Gastroenterology and Urology
Omaha, NE 68197, United States

March 17-20, 2012
Mayo Clinic Gastroenterology and Hepatology
Orlando, FL 32808, United States

March 26-27, 2012
26th Annual New Treatments in Chronic Liver Disease
San Diego, CA 92121, United States

March 30-April 2, 2012
Mayo Clinic Gastroenterology and Hepatology
San Antonio, TX 78249, United States

March 31-April 1, 2012
27th Annual New Treatments in Chronic Liver Disease
San Diego, CA 92121, United States

April 8-10, 2012
9th International Symposium on Functional GI Disorders
Milwaukee, WI 53202, United States

April 13-15, 2012
Asian Oncology Summit 2012
Singapore, Singapore

April 15-17, 2012
European Multidisciplinary Colorectal Cancer Congress 2012
Prague, Czech

April 18-20, 2012
The International Liver Congress 2012
Barcelona, Spain

April 19-21, 2012
Internal Medicine 2012
New Orleans, LA 70166, United States

April 20-22, 2012
Diffuse Small Bowel and Liver Diseases
Melbourne, Australia

April 22-24, 2012
EUROSON 2012 EFSUMB Annual Meeting
Madrid, Spain

April 28, 2012
Issues in Pediatric Oncology
Kiev, Ukraine

May 3-5, 2012
9th Congress of The Jordanian Society of Gastroenterology
Amman, Jordan

May 7-10, 2012
Digestive Diseases Week
Chicago, IL 60601, United States

May 17-21, 2012
2012 ASCRS Annual Meeting-American Society of Colon and Rectal Surgeons
Hollywood, FL 1300, United States

May 18-19, 2012
Pancreas Club Meeting
San Diego, CA 92101, United States

May 18-23, 2012
SGNA: Society of Gastroenterology Nurses and Associates Annual Course
Phoenix, AZ 85001, United States

May 19-22, 2012
2012-Digestive Disease Week
San Diego, CA 92121, United States

June 2-6, 2012
American Society of Colon and Rectal Surgeons Annual Meeting
San Antonio, TX 78249, United States

June 18-21, 2012
Pancreatic Cancer: Progress and Challenges
Lake Tahoe, NV 89101, United States

July 25-26, 2012
PancreasFest 2012
Pittsburgh, PA 15260, United States

September 1-4, 2012
OESO 11th World Conference
Como, Italy

September 6-8, 2012
2012 Joint International Neurogastroenterology and Motility Meeting
Bologna, Italy

September 7-9, 2012
The Viral Hepatitis Congress
Frankfurt, Germany

September 8-9, 2012
New Advances in Inflammatory Bowel Disease
La Jolla, CA 92093, United States

September 8-9, 2012
Florida Gastroenterologic Society 2012 Annual Meeting
Boca Raton, FL 33498, United States

September 15-16, 2012
Current Problems of Gastroenterology and Abdominal Surgery
Kiev, Ukraine

September 20-22, 2012
1st World Congress on Controversies in the Management of Viral Hepatitis
Prague, Czech

October 19-24, 2012
American College of Gastroenterology 77th Annual Scientific Meeting and Postgraduate Course
Las Vegas, NV 89085, United States

November 3-4, 2012
Modern Technologies in Diagnosis and Treatment of Gastroenterological Patients
Dnepropetrovsk, Ukraine

November 4-8, 2012
The Liver Meeting
San Francisco, CA 94101, United States

November 9-13, 2012
American Association for the Study of Liver Diseases
Boston, MA 02298, United States

December 1-4, 2012
Advances in Inflammatory Bowel Diseases
Hollywood, FL 33028, United States

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ic 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 USA* 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 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

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 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

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 DOI:10.1046/j.1526-4610.42.s2.7.x]

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 DOI:10.1097/00003086-200208000-00026]

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9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

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Chapter in a book (list all authors)

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Author(s) and editor(s)

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Conference proceedings

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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)

15 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/index.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

Statistical data

Write as mean ± SD or mean ± SE.

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