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EDITORIAL

Non-alcoholic fatty liver disease and the metabolic syndrome: Effects of weight loss and a review of popular diets. Are low carbohydrate diets the answer?

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Abstract

Non-alcoholic fatty liver disease (NAFLD) encompasses a wide spectrum of fat-induced liver injury, ranging from relatively benign steatosis to cirrhosis and liver failure. The presence of obesity and insulin resistance is strongly associated with non-alcoholic fatty liver and confers on it a greater risk of histologically advanced disease. There is a growing concern in the medical profession as the prevalence of this disease continues to rise in parallel with the rise in obesity and the metabolic syndrome. Treatment options are limited and dietary weight loss is often advised. Low fat diets are difficult to adhere to and recent studies have shown the potential of low carbohydrate diets for weight loss and improving insulin resistance. Thus far, no study has evaluated the effect of low carbohydrate diets on NAFLD. Future studies will be required to address this question and others with regards to the nutritional adequacy and long-term side effects of these diets.

Key words: Non-alcoholic fatty liver disease; Obesity; Metabolic syndrome; Diet management

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INTRODUCTION

In the 1970s, patients undergoing jejunoileal bypass surgery for morbid obesity were noted to develop steatohepatitis and even liver failure following rapid weight loss[1]. Their liver histology was similar to that seen in alcoholics, with macrovesicular steatosis, Mallory hyaline, focal hepatocyte necrosis, mixed lobular inflammation and fibrosis[2]. Similar findings were later described in obese patients without significant alcohol consumption. In 1980, Ludwig *et al*[3] coined the term 'non-alcoholic steatohepatitis' (NASH) to describe these findings. Since then, interest in the disease has grown exponentially in keeping with its rising prevalence. NASH was initially thought to be a benign condition largely limited to middle-aged obese women with diabetes. However, recent studies have shown it to be a far more complex disease that is found in men, women and even children. The spectrum of disease ranges from pure steatosis alone to NASH with hepatic fibrosis, cirrhosis, hepatocellular carcinoma, liver failure and even death[4-6]. Estimates suggest that 20-30% of adults in Western countries may have NAFLD and about 10% of these individuals meet criteria for diagnosis of NASH[7]. NAFLD is now recognized as the most common cause of chronically elevated liver transaminases[8,9] and may be the most common liver disorder[10]. Obesity is the single most common condition found in association with NAFLD. Other features of the metabolic syndrome, such as hyperinsulinemia, hypertriglyceridemia and hypertension, also play a significant pathophysiologic role in its development.

In general, NAFLD in the absence of NASH is an indolent disease with a benign course. However, as noted, end stage liver disease may occur as a consequence of NASH. The seriousness of this condition is demonstrated by the fact that approximately 50% of patients develop fibrosis, 15% develop cirrhosis and 3% may advance to liver failure requiring transplantation[11]. NASH is now being recognized as the underlying cause of most cases of cryptogenic cirrhosis[12,13]. The natural history of NAFLD is poorly understood, and it is not known why some patients progress to cirrhosis, while others do not. However, obesity and insulin resistance have been shown to be associated with more histological advanced disease.

The aim of this article is to review the role of the metabolic syndrome, especially insulin resistance and obesity in the development of NAFLD, to discuss the effect of weight loss on NAFLD and, finally, to evaluate popular diets and compare them with regard to their effects on the metabolic syndrome and NAFLD.

NASH AND METABOLIC SYNDROME

Fatty liver is commonly associated with obesity and insulin resistance. The increasing incidence of NAFLD closely parallels these conditions. There is abundant data showing a relationship between obesity and NAFLD. Wanless *et al*[14] in an autopsy study of 351 patients found that 70% of obese patients had liver steatosis, and the degree of steatosis was proportional to the degree of obesity. The authors also found steatohepatitis in 18.5% and severe fibrosis in 13.8% of markedly obese patients, compared to steatohepatitis in 2.7% and severe fibrosis in 6.6% of lean people. A prospective study performed by Klain *et al*[15] evaluated liver biopsies from 100 consecutive morbidly obese patients undergoing Roux-en-Y gastric bypass. Histological abnormalities were found in 98% of biopsies, and ranged from mild fatty infiltration through inflammatory change to fibrosis and cirrhosis[15]. Data from 90 patients with NASH demonstrated insulin resistance in 85% of them[16]. An Italian study evaluated the risk factors associated with hepatic steatosis. A total of 257 participants were assigned to one of four categories: Controls, teetotalers with normal body mass index (BMI); obese teetotalers; heavy drinkers (> 60 g of alcohol per day) with normal BMI; and heavy drinkers with obesity. The prevalence of steatosis on ultrasound increased from 16% in controls to 46% in heavy drinkers, 76% in obese individuals and 95% in patients with both obesity and heavy alcohol intake. Compared with controls, steatosis was more common by 2.8-fold in heavy drinkers, 4.6-fold in obese persons and 5.8 fold in obese heavy drinkers. In heavy drinkers, obesity increased the risk of steatosis 2.0-fold, while heavy drinking was associated with only a 1.0-fold increased risk in obese subjects[17]. The authors concluded that steatosis was more strongly associated with obesity than with heavy drinking.

Evidence of an etiologic association between NAFLD and metabolic syndrome (hyperglycemia, central obesity, hypertension, hypertriglyceridemia and low HDL-cholesterol) has been shown in both obese and non-obese patients[18]. Studies also have shown that patients with NASH are more insulin resistant than patients with fatty liver alone[19]. Given the wealth of data supporting it, many researchers now consider NAFLD to be a hepatic manifestation of the metabolic syndrome, instead of a primary liver disease[16,20]. Chitturi *et al*[21] tested the hypothesis that insulin resistance is an essential requirement for the development of NASH, and that a high association between insulin resistance and liver disease is relatively specific for NASH. Sixty-six patients with NASH were studied. Insulin resistance was found in virtually all patients (98%) and was seen in both lean and overweight patients. A subset of 36 patients with less severe NASH were compared to 36 age- and sex-matched patients with chronic hepatitis C. The prevalence of insulin resistance was significantly higher in those with NASH than in comparable cases of HCV (75% *vs* 8.3%)[21]. Marchesini *et al*[19] studied liver biopsies in patients with NAFLD. Based on histology, these were classified as having NASH *vs* pure fatty liver. The investigators found that 88% of patients with NASH had metabolic syndrome compared with 53% in patients with pure fatty liver[19]. Marceau *et al*[22] investigated the relationship between liver pathology and the metabolic syndrome. Five hundred fifty one severely obese patients undergoing anti-obesity surgery were studied. Steatosis was found in 86%, fibrosis in 74%, steatohepatitis in 24% and unexpected cirrhosis in 2%. With each addition of the components of metabolic syndrome, the risk of steatosis increased exponentially from 1- to 99-fold[22]. In a series of 505 severely obese patients evaluated before gastroplasty, prevalence of steatosis was significantly higher in patients with impaired glucose tolerance or type II diabetes as compared with non-diabetics. The severity of steatosis was positively correlated with BMI, fasting plasma glucose, insulin and triglyceride concentrations, as well as serum ALT, AST and GGT levels[23].

Issues regarding the nature of hyperinsulinemia in NASH have been raised. It has been questioned as to whether hyperinsulinemia and insulin resistance occur as part of the metabolic syndrome or whether liver damage itself leads to chronic hyperinsulinemia and insulin resistance from impaired insulin degradation, as is seen in cirrhosis. Chitturi *et al*[21] compared the patients with NASH and mild or absent fibrosis with age- and sex-matched patients with HCV, and found that the patients with NASH showed more attributes of insulin resistance than the controls. They had much higher levels of insulin resistance, serum insulin and C-peptide levels. However, the serum C-peptide/insulin ratio was similar in both groups[21]. Pagano *et al*[24] addressed the same question, comparing 19 patients with histologically mild NASH, who had functionally competent livers with 19 normal subjects. Patients with NASH showed marked hyperinsulinemia and insulin resistance as compared with controls, however, the hepatic insulin extraction was similar in both groups[24]. These two studies showed that insulin hypersecretion, and not just impaired insulin degradation, was the basis for hyperinsulinemia in NASH.

The overall incidence of NASH in the severely obese is reported to range from 25-36.4%[25-27]. The prevalence of obesity in the Western world has shown a large increase in the last 20 years. The data of the National Health and Nutrition Examination Survey (NHANES II, 1976-1980) showed a prevalence of 14.5%. By NHANES III (1988-1994), this number had increased to 22.5%, and the data of NHANES 1999-2000 showed a prevalence of 30.5%[28]. Significantly, this number could reach 40% by the year 2025[29]. A similar increase in the number of patients with type 2 diabetes is expected. By some estimates, 29 million people or 7.2% of the population will have type 2 diabetes by the year 2050[30]. Of grave concern is the increasing incidence of obesity in children and adolescents. Given these statistics, the incidence of NASH will rise significantly in the coming years and so will hepatic complications arising from it.

Factors responsible for the development of NAFLD in obese patients are not clear, and the exact mechanism of its progression to fibrosis and cirrhosis has yet to be elucidated. However, our understanding of disease pathogenesis has advanced significantly. Insulin resistance is thought to be a primary pathophysiologic mechanism

in development of fatty liver. Current understanding of the pathogenesis is as follows: Insulin resistance and visceral obesity lead to a hepatic influx of free fatty acids, resulting in increased triglyceride synthesis and decreased triglyceride export. This leads to hepatic steatosis. At this stage, patients have the relatively benign condition of NAFLD. Some of these patients will go on to steatohepatitis. It is unclear why only a small fraction will advance to NASH and what is the exact impetus for this advance. One proposal is that these lipid-laden hepatocytes are susceptible to a "second-hit"[31]. The exact mechanism of this second-hit is unknown. In NASH, as in alcoholic hepatitis, oxidative stress and lipid peroxidation have emerged as the most likely candidates. This "hit" occurs via increased mitochondrial beta-oxidation of the free fatty acids, production of reactive oxygen species and depletion of antioxidants glutathione and vitamin E. This depletion of anti-oxidants hampers reactive oxygen species inactivation, and increases the deleterious effects on the mitochondria. Oxidative stress also results in abnormal cytokine production, especially TNF-α through up-regulation of nuclear translocation of transcription factor nuclear factor κB. This combination of lipid peroxidation and cytokine production results in hepatocyte death.

Another proposed mechanism of development of NASH includes a primary mitochondrial abnormality, as proposed by Sanyal *et al*[32]. This defect, otherwise clinically silent, leads to increased mitochondrial beta oxidation and production of reactive oxygen species in the presence of insulin resistance.

Yang *et al*[33] have demonstrated that obesity itself may cause progression to steatohepatitis by causing Kupffer cell dysfunction and sensitizing the hepatocytes to endotoxin, suggesting that the progression of liver disease may depend on the extent of fatty infiltration[33].

Iron, a strong oxidative agent, has also been proposed as a factor causing the second-hit. Elevated serum ferritin and insulin resistance on those levels have been noted in patients with NASH, as well as increased prevalence of C282Y and H63D mutations in the HFE gene[34,35]. However, evidence that hepatic insulin resistance plays a significant role in fibrosis was found in only one study, and recent studies suggested that increased ferritin levels were likely markers of severe histologic damage and not iron overload[36]. Leptin production by activated hepatic stellate cells has also been considered an important factor in the progression of fatty liver disease and development of fibrosis[37]. Supporting evidence is furnished by genetically leptin-deficient ob/ob mice, which do not develop fibrosis even when fed a methionine-choline-deficient diet.

OBESITY AND INSULIN RESISTANCE AS PREDICTORS OF FIBROSIS

The natural history of NAFLD is not well known, but it is known that prognosis varies according to histologic type. Matteoni *et al*[5] conducted a retrospective study to compare clinical characteristics and outcomes of patients with different types of NAFLD. Patients were separated into four histologic types: Simple fatty liver; steatohepatitis; steatonecrosis; and steatonecrosis plus either Mallory hyaline or fibrosis. Cirrhosis and liver-related death were seen almost exclusively in patients with steatonecrosis with or without Mallory hyaline or fibrosis[5]. The study also confirmed that the prognosis of simple steatosis is favorable.

A number of risk factors for more histologically advanced disease have been identified. Theses include central weight distribution and metabolic syndrome. Dixon *et al*[38] studied 105 severely obese individuals undergoing bariatric surgery, and showed that hyperinsulinemia and increased insulin resistance were associated with adverse histologic findings. The study found that C-peptide was the best predictor of advanced fibrosis (stage 3-4) and that patients with advanced fibrosis had significantly higher C-peptide levels. The insulin resistance index and systemic hypertension were independently associated with advanced NAFLD. Insulin resistance was found to be the best predictor of zone 3-centric steatosis, inflammation and fibrosis. Interestingly, this study found that moderate alcohol consumption reduced the risk of NAFLD by decreasing insulin resistance. Angulo *et al*[39] studied liver biopsies from 144 patients with NASH. In multivariate analysis, older age (> 45 years), obesity, diabetes mellitus and an AST/ALT ratio > 1 were significant predictors of severe fibrosis (bridging/cirrhosis). The investigators concluded that this subgroup would benefit most from liver biopsy and investigational therapies[39]. Researchers in France investigated 93 obese patients, and found that age $\geqslant$ 50 years, BMI > 28 kg/m^2, triglycerides >1.7 mmol/L and ALT > 2 times normal value were independently associated with septal fibrosis[40]. A univariate analysis showed that diabetes and impaired glucose tolerance were significantly associated with fibrosis. Another recent study evaluating steatosis in chronic hepatitis C found that an increased BMI had a role in pathogenesis of steatosis in chronic hepatitis C, and that this may contribute to fibrosis[41]. Studies by both Marceau *et al*[22] and Willner *et al*[16] showed that patients with cirrhosis were more obese than those without cirrhosis. In addition, Marceau *et al*[22] found the presence of diabetes to be the strongest predictor of cirrhosis. Finally, a recent investigation of NAFLD and the metabolic syndrome showed that the presence of metabolic syndrome carried a high risk for NASH among NAFLD patients, and was also associated with a high risk of severe fibrosis[19].

Thus, features of the metabolic syndrome like obesity, insulin resistance and hypertriglyceridemia are not only predisposing factors for NASH, but are also risk factors for more severe fibrosis and advanced disease.

TREATMENT OPTIONS

In light of the increasing incidence of NAFLD-associated comorbid conditions and NAFLD itself, as well as increased awareness of adverse outcomes associated with steatohepatitis, a number of treatment options are being explored. These combine specific therapies for NAFLD as well as the management of comorbid conditions. Therapies that have been evaluated include lifestyle changes such as

diet and exercise, antioxidants like vitamin E and betaine, cytoprotective agents such as ursodeoxycholic acid, lipid-lowering agents, anti-diabetics, weight-loss agents like orlistat and iron reduction therapy, i.e. phlebotomy. The management of associated conditions, such as diabetes, obesity and hyperlipidemia, is especially important, given their association with more advanced liver disease. This may be achieved by optimizing medical treatment of these conditions, as well as through weight loss strategies.

EFFECT OF WEIGHT LOSS ON NAFLD

Because obesity is the most commonly associated condition with NAFLD, weight loss has traditionally been the most commonly suggested intervention. Patients are encouraged to lose weight through exercise and dietary fat restriction. Exercise is of great value as it reduces weight by preferentially decreasing visceral obesity while preventing the loss of muscle mass. It also enhances muscle insulin sensitivity even in the absence of weight loss. A number of studies suggest that NAFLD may improve after weight loss. Improvement in liver biochemistry and ultrasonographic appearance is a consistent finding with moderate weight reduction. However, serum aminotransferases are unreliable markers for follow-up, and do not provide accurate data on prognosis. Worsening of fibrosis can occur even as the levels of transaminases decline[42]. A few studies have evaluated and shown histologic improvement.

The effects of weight reduction on hepatic tests and physical findings were studied in a retrospective review of thirty-nine obese patients without primary liver disease. A weight loss of >10% corrected abnormal liver tests, decreased hepatosplenomegaly and resolved some stigmata of liver disease[43]. Early case series showed that improved liver chemistry and histology was evident with even a modest reduction in weight, as considerable extra weight persisted in the subjects under evaluation[44,45].

Drenick *et al*[46] studied liver biopsies of 41 obese patients undergoing massive weight reduction (>100 lb). Biopsies were obtained at various stages before, during and after weight loss. Based on the method of weight loss, patients were divided into three groups, including prolonged fasting, low-calorie dieting and intestinal bypass surgery. In the non-surgical groups, a transient increase in hepatocellular degeneration and focal necrosis was noted along with progressive diminution of fatty infiltration during weight loss. However, late biopsies revealed normal histology. In patients who underwent intestinal bypass, biopsies variously revealed massive fatty changes, cholestasis, polynuclear inflammatory infiltrates, diffuse fibrosis, bile-duct proliferation and fatal hepatic necrosis[46]. These findings relating to jejunoileal bypass have been demonstrated in several studies[47-49]. This surgical procedure has been abandoned in favor of safer weight-loss surgery.

In a study of twenty-five obese Japanese subjects[50], fifteen underwent a program of restricted diet and exercise for a period of three months, while ten did not. Patients in the intervention group showed a significant decrease in BMI, aminotransferases, total protein, cholinesterase, total cholesterol and fasting plasma glucose levels. In addition, steatosis was significantly improved on liver biopsy in these patients. The ten patients in the control group did not show any change in biochemical parameters or liver histology.

Regular body weight and biological measurements were obtained from 505 severely obese patients before and after undergoing gastroplasty[24]. There was a high prevalence of biologic abnormalities associated with the metabolic syndrome at baseline. After a mean follow-up of 21 ± 14 mo post-surgery, significant reductions in biological markers of metabolic syndrome, such as blood glucose, insulin, and triglycerides, were noted. Also, total cholesterol, uric acid and fibrinogen and ALT levels were reduced, along with an increase in HDL cholesterol levels. Data on children with NASH, who underwent weight reduction, also showed improvement in biochemical and ultrasonographic findings[51,52].

The effect of weight loss on NAFLD was studied in 36 obese patients. Paired liver biopsies were obtained, the first at the time of laparoscopic adjustable gastric band placement and the second after weight reduction. Initial biopsies showed steatosis alone in 12 patients and NASH in 23 patients. Initial fibrosis score of 2 or more was noted in 18 patients. Follow-up biopsies were obtained at 25.6 ± 10 mo after surgery. Weight loss resulted in a significant improvement in liver histology, with repeat biopsies showing NASH in only 4 patients and a fibrosis score of 2 or more in only 3 patients. Greater improvement was seen in patients who had been diagnosed with metabolic syndrome prior to surgery[53].

Knobler *et al*[54] suggested that NAFLD is not only associated with biochemical abnormalities of the metabolic syndrome, but it responds to their amelioration. Forty-eight patients with chronically elevated liver enzymes with clinical, ultrasound, and histologic findings consistent with fatty liver were evaluated. Most of the patients were overweight or obese (64%), 44% had diabetes mellitus, 29% had impaired glucose tolerance, and 17% were hyperinsulinemic. Dietary intervention was the primary mode of weight loss. This was supplemented by oral hypoglycemic or lipid-lowering drugs as needed. The results showed moderate weight loss (3.7 kg), improvement in fasting plasma glucose and lipid levels. An improvement in liver enzymes was noted in 96% patients with normalization in 50% patients.

In a recent pilot study, ten obese patients with NASH were treated with orlistat in addition to diet for weight reduction over a period of 6 mo[55]. BMI, liver enzymes, hemoglobin A1c, fasting lipids, glucose and liver histology were assessed at baseline and at completion of the study. Mean weight loss was 22.7 lb. There was a significant decrease in the BMI, and levels of hemoglobin A1c, ALT, AST. Steatosis improved in six patients and fibrosis in three patients. Hickman *et al*[56] found that modest weight loss through exercise and dietary intervention resulted in sustained improvements not only of ALT and fasting insulin levels, but also of health-related quality of life.

The above data demonstrates that NAFLD and NASH improve significantly with weight reduction. However, this must be done in a controlled manner over a period

of time, as rapid weight loss may lead to exacerbation of liver disease. This has been shown following drastic weight reduction through diet as well as through bariatric surgery. Forty-one patients who had weight loss on a very-low-calorie diet showed improvement in fatty change on liver biopsy. However, 24% developed portal inflammation or fibrosis. These were patients who underwent a very rapid weight reduction (>1.6 kg per week)[57]. Liver biopsies obtained from patients undergoing gastroplasty for morbid obesity were compared before surgery and after weight loss (mean 32 ± 19 kg). There was a significant decrease in the prevalence of steatosis (38% of patients after weight loss *vs* 83% before). However, an increase in the prevalence of hepatitis was observed after significant weight reduction (26% of biopsies after weight loss *versus* 14% before)[58]. The pathogenesis appears to be massive mobilization of fatty acids from visceral stores, which reach the liver via the portal vein and may be toxic to the liver. Therefore, initial target weight reduction should be 10% of baseline weight and should not exceed 1.6 kg/wk.

POPULAR DIETS

The effects of many popular diets on fatty liver are not known. However, metabolic improvements related to dietary weight reduction may favorably influence NASH. If dietary intervention can positively affect insulin resistance and other features of the metabolic syndrome, it would be important to know which particular diet is most beneficial. Conventional diets usually fall into two main categories. Those that alter the macronutrient composition of the diet and those that limit overall energy intake.

Alterations in macronutrient composition

Diets that promote weight loss by emphasizing their macronutrient make-up, as opposed to caloric intake, include low-fat and low-carbohydrate diets. Low-fat diets are traditionally the most recommended by medical professionals. Over time, these have been shown to be safe, cardio-protective and effective in weight loss. Adherence, however, has been a problem. Energy density of food is an important consideration. This refers to the energy (calories) in a given weight of food. Weight loss from a low-fat diet may be due to the low energy density of the diet. Both carbohydrates and proteins have an energy density of 4 kcal/kg, compared to 9 kcal/kg for fats. Researchers for the National Weight Control Registry found that members who maintained successful weight loss, consumed less energy and a lower percentage of energy from fat when compared to the general population[59]. The low-fat (30%) diet is advocated by the National Cholesterol Education Program and by the American Heart Association.

Low carbohydrate diets have been popular periodically over the last several decades, and are currently undergoing a resurgence. An NPD survey on diet trends in the United States showed that in early 2004 about 9% of the population was on a low carbohydrate diet. These diets limit the composition and/or amount (<100 g/d) of carbohydrates, with an increase in dietary protein and fat. They are marketed as low carbohydrate, high protein diets, although they could also be called low carbohydrate, high fat diets. A diet high in carbohydrates results in an increase in blood glucose, insulin and triglycerides, all of which are risk factors for the development of NAFLD. Carbohydrate restriction leads to ketosis resulting not only in weight loss, but also a decrease in blood glucose, insulin and triglyceride levels. Studies have shown these diets to be effective in short-term weight loss[60,61]. Early weight loss is a result of diuresis associated with ketone and urea nitrogen excretion[62]. However, over time, weight loss is a result of loss of body fat. Proponents believe that these diets have a high satiety level, which make them easier to adhere to. This is very important, as dietary adherence is one of the main challenges faced by dieters. Questions with regard to their nutritional adequacy and long-term effects have been raised. In the short-term, these diets have been found to be safe.

Popular low carbohydrate diets include the Atkins, South Beach and the Zone diets. Originally published by Dr. Robert Atkins in 1972 (Dr. Atkins diet revolution), the Atkins diet is the most popular low carbohydrate diet in the United States. Weight reduction is achieved in four phases. The first phase is an induction diet which limits carbohydrates to 20 g/d, but allows unlimited amounts of fat. In phase two, there is ongoing weight loss with easing of the carbohydrate restriction. Through phases three and four, pre-maintenance and maintenance, individuals determine the amount of carbohydrate they can consume while maintaining their weight loss[63]. The South Beach diet consists of 3 phases, gradually increasing in proportion of carbohydrates and emphasizes good carbohydrates and fats. The Zone diet recommends a low carbohydrate, high protein diet, with macronutrient intake in the 40:30:30 ratio, i.e. 40% calories from carbohydrates, 30% from protein and 30% from fat.

Reduction in energy intake

Studies have found that weight loss on a calorie-restricted diet is due to decreased energy intake and not nutrient composition. Golay *et al*[64] evaluated the effect of diets that were equally low in energy, but widely different in amounts of fat and carbohydrate over a 6-wk period. No significant difference in amount of weight loss was noted[64]. Alford *et al*[65] studied the effects of three different diets with a fixed caloric intake of 1 200 kcal/d. They found no significant difference in weight loss among diets with 25%, 45%, 75% carbohydrate. The authors concluded that weight loss is the result of reduction in caloric intake in proportion to caloric requirements. Schlundt *et al*[66] found greater weight loss with a low-fat, calorie-restricted diet when compared to a low-fat, *ad libitum* carbohydrate intake diet. A systematic review of low carbohydrate diets by Freedman *et al*[67] found weight loss to be associated with energy restriction and not carbohydrate restriction. It is likely that individuals on diets that alter macronutrient composition (low fat or low carbohydrate) actually lose weight because of a concurrent reduction in energy intake.

LOW-CARBOHYDRATE *VERSUS* LOW-FAT DIETS: EFFECTS ON BIOCHEMICAL MARKERS OF METABOLIC SYNDROME AND NAFLD

As mentioned previously, dietary weight reduction has been shown to have a positive effect on biochemical markers of the metabolic syndrome. This may translate into a positive effect on NAFLD. Encouraging histologic findings have been noted in these patients. Therefore, it is important to compare the various diets and determine which, if any, is most beneficial in NAFLD.

Low-carbohydrate diets have long been considered fad diets by the medical profession. Several questions have been raised regarding their weight loss potential and possible adverse effects. Public interest continues unabated, and books detailing low carbohydrate lifestyles are regulars on best seller lists. A number of studies have been published in recent years evaluating the effects of low-carbohydrate diets on weight loss as well as on metabolic markers, comparing these diets to traditional low-fat diets. However, none has compared their effects on liver histology and NAFLD. Effects on obesity and biomarkers of the metabolic syndrome will be reviewed, given their important etiologic association with NAFLD.

In an uncontrolled study, Westman *et al*[68] showed that in mildly obese, motivated persons, a very low-carbohydrate diet led to sustained weight loss during a 6-mo period. Positive effects were also noted on the serum lipid profile, with a decrease in triglycerides, total and low-density lipoprotein cholesterol and an increase in high-density lipoprotein cholesterol levels. More recently, several randomized controlled trials comparing various diets have been published. Brehm *et al*[69] studied the effects of a very low-carbohydrate diet on body composition and cardiovascular risk factors. Fifty three healthy, obese women were randomized to 6 mo of an *ad libitum* very low-carbohydrate diet *versus* a calorie-restricted low-fat diet. The very low carbohydrate group had more weight loss (8.5 ± 1.0 *vs* 3.9 ± 1.0 kg, $P<0.001$) without any deleterious effect on cardiovascular risk factors. Foster *et al* randomized 63 non-diabetic obese subjects to a low carbohydrate (Atkins) *versus* a conventional diet (low fat, high carbohydrate, calorie-restricted diet). The Atkins group had more weight loss at 3 mo (-6.8 ± 5.0 % *vs* -2.7 ± 3.7%) and 6 mo (-7.0 ± 6.5% *vs* -3.2 ± 5.6%), but at 12 mo the difference between the 2 groups was not significant (-4.4 ± 6.7% *vs* -2.5 ± 6.3%). The low carbohydrate group was associated with a greater decrease in triglycerides and a greater increase in high-density lipoprotein cholesterol levels. No difference in the total of low-density lipoprotein cholesterol levels was seen between the both groups. Insulin sensitivity increased in both groups without any significant difference between groups[61]. In a study by Samaha *et al*[60], 132 severely obese subjects with a mean body mass index (BMI) of 43 (diabetes mellitus in 39% and metabolic syndrome in 43%) were randomized to a low-carbohydrate or a low-fat, calorie-restricted diet. At 6 mo, the low carbohydrate group had more weight loss (-5.8 ± 8.6 kg *vs* -1.9 ± 4.2 kg) and a greater decrease in triglyceride levels (-20 ± 43% *vs* -4 ± 31%). In non-diabetics, the insulin sensitivity improved more, and in diabetics, fasting glucose levels decreased more in the low carbohydrate group[60]. No adverse effects on the serum lipid levels were observed. The 12-mo data from this study was published in 2004. Weight loss between the two groups at this point was no longer significant. The favorable metabolic response to the low-carbohydrate diet, however, persisted[70]. A recently published study of 120 overweight, hyperlipidemic subjects showed similar findings. At 24 wk, the low carbohydrate (Atkins) group had greater weight loss (mean -12.9% *vs* -6.7%, $P<0.001$). Effects on triglyceride and high-density lipoprotein cholesterol levels were also more favorable with the low-carbohydrate diet[71]. Comparison of the National Cholesterol Education Program (NCEP) diet with a modified low-carbohydrate diet (low in total carbohydrates but higher in complex carbohydrates, protein and monounsaturated fat) showed a significantly greater weight loss in the modified low-carbohydrate diet over a period of 12 wk. This study did not show any significant difference between groups in blood lipid levels[72]. A low-carbohydrate diet over 12 wk was also found to be more effective than a low-fat diet for weight loss in overweight adolescents without adversely affecting their lipid profile[73]. In comparing 4 popular diets (Atkins, Zone, Weight Watcher's and Ornish) over one year, all were found to reduce weight modestly, and to have a significant reduction in low-density lipoprotein/high-density lipoprotein cholesterol ratio. No significant difference was noted between diets, and no diet-related adverse effects were noted[74]. Evaluation of 3 different diets in overweight, insulin-resistant women showed greater weight reduction from the Atkins and Zone diets when compared to a conventional low-fat diet. A greater reduction in triglycerides and waist circumference was also noted with these diets. A significant increase in low-density lipoprotein levels was noted in 25% of subjects on the Atkins diet, whereas this was seen in only 13% of subjects on the low-fat diet and 3% of those on the Zone diet[75]. Improvement in characteristics of the metabolic syndrome as demonstrated by a decrease in triglycerides, triglyceride/high-density lipoprotein ratio, postprandial lipemia and increase in low-density lipoprotein particle size was shown in overweight men on a very low-carbohydrate diet[76]. Similarly, improved insulin sensitivity and prevention of HDL cholesterol decline were noted in overweight men[77].

Results from these studies suggest that a low-carbohydrate diet results in weight loss and may even be more effective than a conventional, low-fat diet in the short-term period. Greater weight loss may be the result of the monotony and simplicity of the diet inhibiting appetite and food intake[78]. Enhanced satiety, palatability and novelty of the diet may also play a role[60,70]. There has been great concern regarding negative effects on renal function, bone health and cancer risk. Some studies showed that low-carbohydrate diets had a higher incidence of minor side-effects, such as constipation, headache, halitosis, muscle cramps, diarrhea, rash and general weakness[71]. The major concern of an adverse effect on serum lipids, renal and cardiovascular health was not realized in these

studies. Again, these studies have relatively short follow-up periods, and the effect on low-density lipoprotein cholesterol levels remains uncertain, and requires further study. Caution must be exercised in subjects with baseline abnormal low-density lipoprotein cholesterol levels. Effects on biochemical markers associated with the metabolic syndrome appear to be more favorable with low-carbohydrate diets. In general, these diets show greater improvements in insulin sensitivity, triglyceride and high-density cholesterol levels. It is possible that for patients with the metabolic syndrome, a low-carbohydrate diet may be more advantageous. This, in turn, may positively affect NAFLD.

CONCLUSION

As the prevalence of obesity increases, so will that of the metabolic syndrome and NAFLD. It is now recognized that the consequences of NAFLD are not always benign. While pure steatosis alone is generally an indolent disease, steatohepatitis can be a progressive disease leading to cirrhosis and even liver failure. The etiologic association between NAFLD and the metabolic syndrome is so well established that NAFLD is considered a hepatic manifestation of the disease. Obesity and insulin resistance are associated with more histologically advanced disease. Given this scenario, it is important to develop new strategies to treat and prevent NASH. While it is known that dietary weight loss improves markers of the metabolic syndrome and data also suggest that judicious weight loss affects the liver favorably in NAFLD, the best dietary approach is yet unknown. Traditionally, a low-fat diet has been recommended, but recent studies, show greater short-term weight loss and greater improvement in markers of the metabolic syndrome without significant adverse effects with low-carbohydrate diets. This raises the question of whether low-carbohydrate diets should be recommended as part of a weight loss strategy for our patients. At this point, questions regarding the nutritional adequacy and long-term safety remain. While studies have evaluated the effect of these diets on weight loss, cardiovascular and metabolic marker studies are needed to evaluate the effect of these diets specifically on NAFLD.

REFERENCES

1 **McGill DB**, Humpherys SR, Baggenstoss AH, Dickson ER. Cirrhosis and death after jejunoileal shunt. *Gastroenterology* 1972; **63**: 872-877
2 **Peters RL**, Gay T, Reynolds TB. Post-jejunoileal-bypass hepatic disease. Its similarity to alcoholic hepatic disease. *Am J Clin Pathol* 1975; **63**: 318-331
3 **Ludwig J**, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434-438
4 **Bacon BR**, Farahvash MJ, Janney CG, Neuschwander-Tetri BA. Nonalcoholic steatohepatitis: an expanded clinical entity. *Gastroenterology* 1994; **107**: 1103-1109
5 **Matteoni CA**, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413-1419
6 **Bugianesi E**, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140
7 **Neuschwander-Tetri BA**, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202-1219
8 **Clark JM**, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol* 2003; **98**: 960-967
9 **Daniel S**, Ben-Menachem T, Vasudevan G, Ma CK, Blumenkehl M. Prospective evaluation of unexplained chronic liver transaminase abnormalities in asymptomatic and symptomatic patients. *Am J Gastroenterol* 1999; **94**: 3010-3014
10 **Mathiesen UL**, Franzén LE, Frydén A, Foberg U, Bodemar G. The clinical significance of slightly to moderately increased liver transaminase values in asymptomatic patients. *Scand J Gastroenterol* 1999; **34**: 85-91
11 **Sheth SG**, Gordon FD, Chopra S. Nonalcoholic steatohepatitis. *Ann Intern Med* 1997; **126**: 137-145
12 **Caldwell SH**, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999; **29**: 664-669
13 **Poonawala A**, Nair SP, Thuluvath PJ. Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study. *Hepatology* 2000; **32**: 689-692
14 **Wanless IR**, Lentz JS. Fatty liver hepatitis (steatohepatitis) and obesity: an autopsy study with analysis of risk factors. *Hepatology* 1990; **12**: 1106-1110
15 **Klain J**, Fraser D, Goldstein J, Peiser J, Avinoah E, Ovnat A, Charuzi I. Liver histology abnormalities in the morbidly obese. *Hepatology* 1989; **10**: 873-876
16 **Willner IR**, Waters B, Patil SR, Reuben A, Morelli J, Riely CA. Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol* 2001; **96**: 2957-2961
17 **Bellentani S**, Saccoccio G, Masutti F, Crocè LS, Brandi G, Sasso F, Cristanini G, Tiribelli C. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000; **132**: 112-117
18 **Marchesini G**, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 1999; **107**: 450-455
19 **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
20 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; **50**: 1844-1850
21 **Chitturi S**, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, Karim R, Lin R, Samarasinghe D, Liddle C, Weltman M, George J. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology* 2002; **35**: 373-379
22 **Marceau P**, Biron S, Hould FS, Marceau S, Simard S, Thung SN, Kral JG. Liver pathology and the metabolic syndrome X in severe obesity. *J Clin Endocrinol Metab* 1999; **84**: 1513-1517
23 **Luyckx FH**, Scheen AJ, Desaive C, Dewe W, Gielen JE, Lefebvre PJ. Effects of gastroplasty on body weight and related biological abnormalities in morbid obesity. *Diabetes Metab* 1998; **24**: 355-361
24 **Pagano G**, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, Cassader M, David E, Cavallo-Perin P, Rizzetto M. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. *Hepatology* 2002; **35**: 367-372
25 **Braillon A**, Capron JP, Hervé MA, Degott C, Quenum C. Liver in obesity. *Gut* 1985; **26**: 133-139
26 **Silverman JF**, O'Brien KF, Long S, Leggett N, Khazanie PG, Pories WJ, Norris HT, Caro JF. Liver pathology in morbidly

obese patients with and without diabetes. *Am J Gastroenterol* 1990; **85**: 1349-1355
27 **Abrams GA**, Kunde SS, Lazenby AJ, Clements RH. Portal fibrosis and hepatic steatosis in morbidly obese subjects: A spectrum of nonalcoholic fatty liver disease. *Hepatology* 2004; **40**: 475-483
28 **Flegal KM**, Carroll MD, Ogden CL, Johnson CL. Prevalence and trends in obesity among US adults, 1999-2000. *JAMA* 2002; **288**: 1723-1727
29 **Kopelman PG**. Obesity as a medical problem. *Nature* 2000; **404**: 635-643
30 **Boyle JP**, Honeycutt AA, Narayan KM, Hoerger TJ, Geiss LS, Chen H, Thompson TJ. Projection of diabetes burden through 2050: impact of changing demography and disease prevalence in the U.S. *Diabetes Care* 2001; **24**: 1936-1940
31 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845
32 **Sanyal AJ**, Campbell-Sargent C, Mirshahi F, Rizzo WB, Contos MJ, Sterling RK, Luketic VA, Shiffman ML, Clore JN. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. *Gastroenterology* 2001; **120**: 1183-1192
33 **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 U S A* 1997; **94**: 2557-2562
34 **George DK**, Goldwurm S, MacDonald GA, Cowley LL, Walker NI, Ward PJ, Jazwinska EC, Powell LW. Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. *Gastroenterology* 1998; **114**: 311-318
35 **Bonkovsky HL**, Jawaid Q, Tortorelli K, LeClair P, Cobb J, Lambrecht RW, Banner BF. Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. *J Hepatol* 1999; **31**: 421-429
36 **Bugianesi E**, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, Massarenti P, Piga A, Marchesini G, Rizzetto M. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology* 2004; **39**: 179-187
37 **Saxena NK**, Ikeda K, Rockey DC, Friedman SL, Anania FA. Leptin in hepatic fibrosis: evidence for increased collagen production in stellate cells and lean littermates of ob/ob mice. *Hepatology* 2002; **35**: 762-771
38 **Dixon JB**, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. *Gastroenterology* 2001; **121**: 91-100
39 **Angulo P**, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999; **30**: 1356-1362
40 **Ratziu V**, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T. Liver fibrosis in overweight patients. *Gastroenterology* 2000; **118**: 1117-1123
41 **Hourigan LF**, Macdonald GA, Purdie D, Whitehall VH, Shorthouse C, Clouston A, Powell EE. Fibrosis in chronic hepatitis C correlates significantly with body mass index and steatosis. *Hepatology* 1999; **29**: 1215-1219
42 **Adams LA**, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; **42**: 132-138
43 **Palmer M**, Schaffner F. Effect of weight reduction on hepatic abnormalities in overweight patients. *Gastroenterology* 1990; **99**: 1408-1413
44 **Keeffe EB**, Adesman PW, Stenzel P, Palmer RM. Steatosis and cirrhosis in an obese diabetic. Resolution of fatty liver by fasting. *Dig Dis Sci* 1987; **32**: 441-445
45 **Eriksson S**, Eriksson KF, Bondesson L. Nonalcoholic steatohepatitis in obesity: a reversible condition. *Acta Med Scand* 1986; **220**: 83-88
46 **Drenick EJ**, Simmons F, Murphy JF. Effect on hepatic morphology of treatment of obesity by fasting, reducing diets and small-bowel bypass. *N Engl J Med* 1970; **282**: 829-834
47 **Ames FC**, Copeland EM, Leeb DC, Moore DL, Dudrick SJ. Liver dysfunction following small-bowel bypass for obesity. Nonoperative treatment of fatty metamorphosis with parenteral hyperalimentation. *JAMA* 1976; **235**: 1249-1252
48 **Kroyer JM**, Talbert WM. Morphologic liver changes in intestinal bypass patients. *Am J Surg* 1980; **139**: 855-859
49 **Kaminski DL**, Herrmann VM, Martin S. Late effects of jejunoileal bypass operations on hepatic inflammation, fibrosis and lipid content. *Hepatogastroenterology* 1985; **32**: 159-162
50 **Ueno T**, Sugawara H, Sujaku K, Hashimoto O, Tsuji R, Tamaki S, Torimura T, Inuzuka S, Sata M, Tanikawa K. Therapeutic effects of restricted diet and exercise in obese patients with fatty liver. *J Hepatol* 1997; **27**: 103-107
51 **Vajro P**, Fontanella A, Perna C, Orso G, Tedesco M, De Vincenzo A. Persistent hyperaminotransferasemia resolving after weight reduction in obese children. *J Pediatr* 1994; **125**: 239-241
52 **Franzese A**, Vajro P, Argenziano A, Puzziello A, Iannucci MP, Saviano MC, Brunetti F, Rubino A. Liver involvement in obese children. Ultrasonography and liver enzyme levels at diagnosis and during follow-up in an Italian population. *Dig Dis Sci* 1997; **42**: 1428-1432
53 **Dixon JB**, Bhathal PS, Hughes NR, O'Brien PE. Nonalcoholic fatty liver disease: Improvement in liver histological analysis with weight loss. *Hepatology* 2004; **39**: 1647-1654
54 **Knobler H**, Schattner A, Zhornicki T, Malnick SD, Keter D, Sokolovskaya N, Lurie Y, Bass DD. Fatty liver--an additional and treatable feature of the insulin resistance syndrome. *QJM* 1999; **92**: 73-79
55 **Harrison SA**, Fincke C, Helinski D, Torgerson S, Hayashi P. A pilot study of orlistat treatment in obese, non-alcoholic steatohepatitis patients. *Aliment Pharmacol Ther* 2004; **20**: 623-628
56 **Hickman IJ**, Jonsson JR, Prins JB, Ash S, Purdie DM, Clouston AD, Powell EE. Modest weight loss and physical activity in overweight patients with chronic liver disease results in sustained improvements in alanine aminotransferase, fasting insulin, and quality of life. *Gut* 2004; **53**: 413-419
57 **Andersen T**, Gluud C, Franzmann MB, Christoffersen P. Hepatic effects of dietary weight loss in morbidly obese subjects. *J Hepatol* 1991; **12**: 224-229
58 **Luyckx FH**, Desaive C, Thiry A, Dewé W, Scheen AJ, Gielen JE, Lefèbvre PJ. Liver abnormalities in severely obese subjects: effect of drastic weight loss after gastroplasty. *Int J Obes Relat Metab Disord* 1998; **22**: 222-226
59 **Shick SM**, Wing RR, Klem ML, McGuire MT, Hill JO, Seagle H. Persons successful at long-term weight loss and maintenance continue to consume a low-energy, low-fat diet. *J Am Diet Assoc* 1998; **98**: 408-413
60 **Samaha FF**, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, Williams T, Williams M, Gracely EJ, Stern L. A low-carbohydrate as compared with a low-fat diet in severe obesity. *N Engl J Med* 2003; **348**: 2074-2081
61 **Foster GD**, Wyatt HR, Hill JO, McGuckin BG, Brill C, Mohammed BS, Szapary PO, Rader DJ, Edman JS, Klein S. A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med* 2003; **348**: 2082-2090
62 **Yang MU**, Van Itallie TB. Composition of weight lost during short-term weight reduction. Metabolic responses of obese subjects to starvation and low-calorie ketogenic and nonketogenic diets. *J Clin Invest* 1976; **58**: 722-730
63 **Atkins RC**. Dr. Atkins New Diet Revolution. New York: Avon Books, 2002: 123-226
64 **Golay A**, Allaz AF, Morel Y, de Tonnac N, Tankova S, Reaven G. Similar weight loss with low- or high-carbohydrate diets. *Am J Clin Nutr* 1996; **63**: 174-178
65 **Alford BB**, Blankenship AC, Hagen RD. The effects of variations in carbohydrate, protein, and fat content of the diet upon weight loss, blood values, and nutrient intake of adult obese women. *J Am Diet Assoc* 1990; **90**: 534-540
66 **Schlundt DG**, Hill JO, Pope-Cordle J, Arnold D, Virts KL, Katahn M. Randomized evaluation of a low fat ad libitum

carbohydrate diet for weight reduction. *Int J Obes Relat Metab Disord* 1993; **17**: 623-629
67 **Freedman MR**, King J, Kennedy E. Popular diets: a scientific review. *Obes Res* 2001; **9 Suppl 1**: 1S-40S
68 **Westman EC**, Yancy WS, Edman JS, Tomlin KF, Perkins CE. Effect of 6-month adherence to a very low carbohydrate diet program. *Am J Med* 2002; **113**: 30-36
69 **Brehm BJ**, Seeley RJ, Daniels SR, D'Alessio DA. A randomized trial comparing a very low carbohydrate diet and a calorie-restricted low fat diet on body weight and cardiovascular risk factors in healthy women. *J Clin Endocrinol Metab* 2003; **88**: 1617-1623
70 **Stern L**, Iqbal N, Seshadri P, Chicano KL, Daily DA, McGrory J, Williams M, Gracely EJ, Samaha FF. The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. *Ann Intern Med* 2004; **140**: 778-785
71 **Yancy WS**, Olsen MK, Guyton JR, Bakst RP, Westman EC. A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: a randomized, controlled trial. *Ann Intern Med* 2004; **140**: 769-777
72 **Aude YW**, Agatston AS, Lopez-Jimenez F, Lieberman EH, Marie Almon M, Rojas G, Lamas GA, Hennekens CH. The national cholesterol education program diet vs a diet lower in carbohydrates and higher in protein and monounsaturated fat: a randomized trial. *Arch Intern Med* 2004; **164**: 2141-2146
73 **Sondike SB**, Copperman N, Jacobson MS. Effects of a low-carbohydrate diet on weight loss and cardiovascular risk factor in overweight adolescents. *J Pediatr* 2003; **142**: 253-258
74 **Dansinger ML**, Gleason JA, Griffith JL, Selker HP, Schaefer EJ. Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. *JAMA* 2005; **293**: 43-53
75 **McAuley KA**, Hopkins CM, Smith KJ, McLay RT, Williams SM, Taylor RW, Mann JI. Comparison of high-fat and high-protein diets with a high-carbohydrate diet in insulin-resistant obese women. *Diabetologia* 2005; **48**: 8-16
76 **Sharman MJ**, Gómez AL, Kraemer WJ, Volek JS. Very low-carbohydrate and low-fat diets affect fasting lipids and postprandial lipemia differently in overweight men. *J Nutr* 2004; **134**: 880-885
77 **Volek JS**, Sharman MJ, Gómez AL, DiPasquale C, Roti M, Pumerantz A, Kraemer WJ. Comparison of a very low-carbohydrate and low-fat diet on fasting lipids, LDL subclasses, insulin resistance, and postprandial lipemic responses in overweight women. *J Am Coll Nutr* 2004; **23**: 177-184
78 **Astrup A**, Meinert Larsen T, Harper A. Atkins and other low-carbohydrate diets: hoax or an effective tool for weight loss? *Lancet* ; **364**: 897-899

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REVIEW

Epidemiology of gastric cancer

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Abstract

The incidence and mortality of gastric cancer have fallen dramatically in US and elsewhere over the past several decades. Nonetheless, gastric cancer remains a major public health issue as the fourth most common cancer and the second leading cause of cancer death worldwide. Demographic trends differ by tumor location and histology. While there has been a marked decline in distal, intestinal type gastric cancers, the incidence of proximal, diffuse type adenocarcinomas of the gastric cardia has been increasing, particularly in the Western countries. Incidence by tumor sub-site also varies widely based on geographic location, race, and socio-economic status. Distal gastric cancer predominates in developing countries, among blacks, and in lower socio-economic groups, whereas proximal tumors are more common in developed countries, among whites, and in higher socio-economic classes. Diverging trends in the incidence of gastric cancer by tumor location suggest that they may represent two diseases with different etiologies. The main risk factors for distal gastric cancer include *Helicobacter pylori* (*H pylori*) infection and dietary factors, whereas gastroesophageal reflux disease and obesity play important roles in the development of proximal stomach cancer. The purpose of this review is to examine the epidemiology and risk factors of gastric cancer, and to discuss strategies for primary prevention.

Key words: Epidemiology; Gastric cancer

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INTRODUCTION

Overall, gastric cancer incidence and mortality have fallen dramatically over the past 70 years[1]. Despite its recent decline, gastric cancer is the fourth most common cancer and the second leading cause of cancer-related death worldwide[2,3]. In 2000, about 880 000 people were diagnosed with gastric cancer and approximately 650 000 died of the disease[4].

The two main tumor sites of gastric adenocarcinoma are proximal (cardia) and distal (noncardia). Despite a decline in distal gastric cancers, proximal tumors have been increasing in incidence since the 1970s, especially among males in the Western countries[5,6]. These gastric tumor types predominate in populations from different geographic locations, racial and socio-economic groups. They may also differ in genetic susceptibility, pathologic profile, clinical presentation, and prognosis. The observed differences between gastric cancers by anatomic site suggest that they are distinct diseases with different etiologies. Detailed epidemiological analyses of their demographic trends and risk factors will help guide future cancer control strategies.

PATHOLOGIC CONSIDERATIONS

About 90% of stomach tumors are adenocarcinomas, which are subdivided into two main histologic types: (1) well-differentiated or intestinal type, and (2) undifferentiated or diffuse type. The intestinal type is related to corpus-dominant gastritis with gastric atrophy and intestinal metaplasia, whereas the diffuse type usually originates in pangastritis without atrophy.

The intestinal type is more common in males, blacks, and older age groups, whereas the diffuse type has a more equal male-to-female ratio and is more frequent in younger individuals[7,8]. Intestinal type tumors predominate in high-risk geographic areas, such as East Asia, Eastern Europe, Central and South America, and account for much of the international variation of gastric cancer[9]. Diffuse type adenocarcinomas of the stomach have a more uniform geographic distribution[10]. A decline in the incidence of the intestinal type tumors in the corpus of the stomach accounts for most of the recent decrease in gastric cancer rates worldwide[11]. In contrast, the incidence of diffuse type gastric carcinoma, particularly the signet ring type, has been increasing[12].

DEMOGRAPHIC TRENDS

Time trends

In the 1930s, gastric cancer was the most common cause of cancer death in US and Europe. During the past 70 years, mortality rates have fallen dramatically

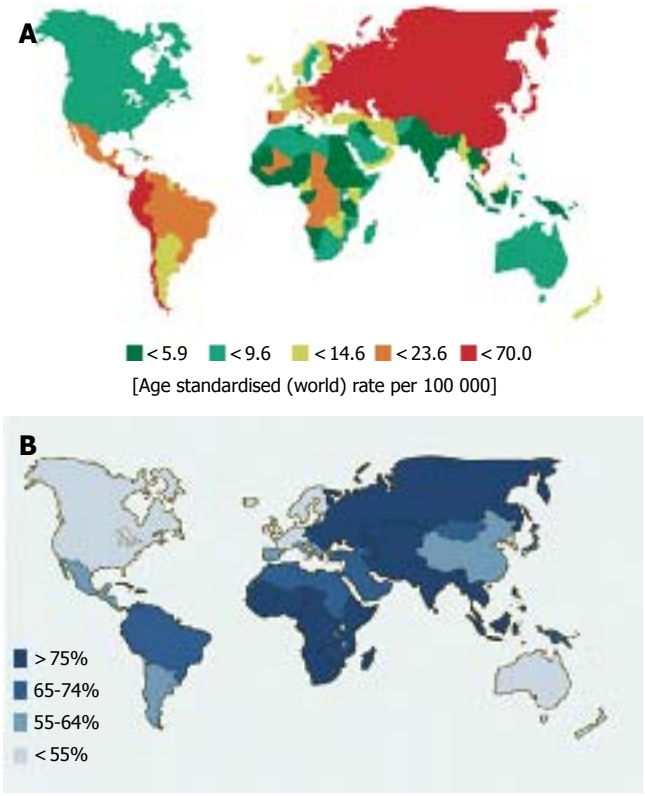

Figure 1 (**A**) Incidence of stomach cancer in males. (**B**) Prevalence of *H pylori* infection in asymptomatic adults. (Data adopted from Parkin *et al*[3].)

in all developed countries largely due to unplanned prevention. However, in the past 30 years, the incidence of gastric cardia adenocarcinoma rose by five- to six-fold in developed countries[13-19]. Gastric cardia tumors now account for nearly half of all stomach cancers among men from US and UK[6,20]. There has also been a rising trend in esophageal adenocarcinoma, in which obesity, gastroesophageal reflux disease (GERD), and Barrett's esophagus are major etiologic factors. Gastric cardia cancers share certain epidemiologic features with adenocarcinomas of the distal esophagus and gastroesophageal (GE) junction, suggesting that they represent a similar disease entity.

Geographic variation

Gastric cancer incidence rates vary by up to ten-fold throughout the world. Nearly two-thirds of stomach cancers occur in developing countries[4]. Japan and Korea have the highest gastric cancer rates in the world[21,22]. High-incidence areas for noncardia gastric adenocarcinoma include East Asia, Eastern Europe, and Central and South America[20,23]. Low incidence rates are found in South Asia, North and East Africa, North America, Australia, and New Zealand (Figure 1A).

In Japan, gastric cancer remains the most common type of cancer among both men and women. Age-standardized incidence rates in Japan are 69.2 per 100 000 in men and 28.6 per 100 000 in women[3]. In contrast to the increasing incidence of proximal tumors in the West, distal tumors continue to predominate in Japan. However, even in Japan, the proportion of proximal stomach cancers has increased among men[24].

Migrant populations from high-risk areas such as Japan show a marked reduction in risk when they move to low-incidence regions such as the US[25]. Subsequent generations acquire risk levels approximating those of the host country[20,23].

Sex, race, and age distribution

Noncardia gastric cancer has a male-to-female ratio of approximately 2:1[20,23]. Incidence rates are significantly higher among blacks and lower socio-economic groups, and in developing countries[20]. Incidence rises progressively with age, with a peak incidence between 50 and 70 years.

In contrast, for gastric cardia carcinomas, men are affected five times more than women and whites twice as much as blacks[26]. In addition, the incidence rates of proximal gastric cancers are relatively higher in the professional classes[27]. Different rates of genetic polymorphisms according to tumor sub-site suggest variation in susceptibility to stomach cancer by tumor location[28]. These findings suggest that noncardia and cardia adenocarcinomas are distinct biological entities.

SURVIVAL

For the past few decades, gastric cancer mortality has decreased markedly in most areas of the world[29,30]. However, gastric cancer remains a disease of poor prognosis and high mortality, second only to lung cancer as the leading cause of cancer-related death worldwide. In general, countries with higher incidence rates of gastric cancer show better survival rates than countries with lower incidence[31]. This association is largely due to a difference in survival rates based on tumor location within the stomach. Tumors located in the gastric cardia have a much poorer prognosis compared to those in the pyloric antrum, with lower 5-year survival and higher operative mortality[32].

In addition, the availability of screening for early detection in high-risk areas has led to a decrease in mortality. In Japan where mass screening programs are in place, mortality rates for gastric cancer in men have more than halved since the early 1970s[33]. When disease is confined to the inner lining of the stomach wall, 5-year survival is on the order of 95%. In contrast, few gastric cancers are discovered at an early stage in US, leading to 5-year relative survival rates of less than 20%[34]. Similarly in European countries, the 5-year relative survival rates for gastric cancer vary from 10% to 20%[35,36]. Host-related factors may also affect prognosis, as a US study demonstrated that gastric cancers in persons of Asian descent had a better prognosis compared to non-Asians[37].

RISK FACTORS

Gastric cancer is a multifactorial disease. The marked geographic variation, time trends, and the migratory effect on gastric cancer incidence suggest that environmental or lifestyle factors are major contributors to the etiology of this disease.

Helicobacter pylori infection

H pylori is a gram-negative bacillus that colonizes the stomach and may be the most common chronic bacterial

infection worldwide[38]. Countries with high gastric cancer rates typically have a high prevalence of *H pylori* infection, and the decline in *H pylori* prevalence in developed countries parallels the decreasing incidence of gastric cancer[39,40] (Figure 1B). In US, the prevalence of *H pylori* infection is <20% at the age 20 years and 50% at 50 years[41]. In Japan, it is also <20% at 20 years, but increases to 80% over the age of 40 years[42] and in Korea, 90% of asymptomatic adults over the age of 20 years are infected by *H pylori*[43]. The increase in prevalence with age is largely due to a birth cohort effect rather than late acquisition of infection. *H pylori* infection is mainly acquired during early childhood, likely through oral ingestion, and infection persists throughout life[44]. Prevalence is closely linked to socio-economic factors, such as low income and poor education, and living conditions during childhood, such as poor sanitation and overcrowding[45-49].

The association between chronic *H pylori* infection and the development of gastric cancer is well established[50-53]. In 1994, the International Agency for Research on Cancer classified *H pylori* as a type I (definite) carcinogen in human beings[54]. In Correa's model of gastric carcinogenesis, *H pylori* infection triggers the progressive sequence of gastric lesions from chronic gastritis, gastric atrophy, intestinal metaplasia, dysplasia, and finally, gastric adenocarcinoma[55]. Several case-control studies have shown significant associations between *H pylori* seropositivity and gastric cancer risk, with about a 2.1- to 16.7-fold greater risk compared to seronegative individuals[56-62]. Prospective studies have also supported the association between *H pylori* infection and gastric cancer risk[50-52,63]. Perhaps the most compelling evidence for the link between *H pylori* and gastric cancer comes from a prospective study of 1 526 Japanese participants in which gastric cancers developed in 2.9% of infected people and in none of the uninfected individuals[64]. Interestingly, gastric carcinomas were detected in 4.7% of *H pylori*-infected individuals with non-ulcer dyspepsia.

The vast majority of *H pylori*-infected individuals remain asymptomatic without any clinical sequelae. Cofactors, which determine that *H pylori*-infected people are at particular risk for gastric cancer, include bacterial virulence factors and proinflammatory host factors. Gastric cancer risk is enhanced by infection with a more virulent strain of *H pylori* carrying the cytotoxin-associated gene A (*cagA*)[65,66]. Compared to *cagA*- strains, infection by *H pylori cagA*+ strains was associated with an increased risk of severe atrophic gastritis and distal gastric cancer[67-70]. In the Western countries, about 60% of *H pylori* isolates are *cagA*+[71], whereas in Japan, nearly 100% of the strains possess functional *cagA*[72,73]. Host factors associated with an increased risk of gastric cancer include genetic polymorphisms which lead to high-level of expression of the proinflammatory cytokine, interleukin-1β[74,75].

The effects of *H pylori* on gastric tumor development may vary by anatomical site. The falling incidence of *H pylori* infection and noncardia gastric cancer in developed countries has been diametrically opposed to the rapid increase in the incidence of gastric cardia adenocarcinoma[76]. Based on a meta-analysis of prospective cohort studies, *H pylori* infection was associated with the risk of noncardia gastric cancer, but not cardia cancer[77]. Other studies demonstrated a significant inverse association between *H pylori* infection, particularly *cagA*+ strains, and the development of gastric cardia and esophageal adenocarcinomas[78,79]. In the Western countries, where the prevalence of *H pylori* infection is falling, GERD and its sequelae are increasing. Studies have shown that severe atrophic gastritis and reduced acid production associated with *H pylori* infection significantly reduced the risk of GERD[80-83]. However, recent studies have found conflicting results on whether *H pylori* eradication therapy increases the risk of esophagitis and gastric cardia adenocarcinoma[84-91]. Thus, the protective effect of *H pylori* against cardia tumors remains controversial.

Dietary factors

It is unlikely that *H pylori* infection alone is responsible for the development of gastric cancer. Rather, *H pylori* may produce an environment conducive to carcinogenesis and interact with other lifestyle and environmental exposures. There is evidence that consumption of salty foods and *N*-nitroso compounds and low intake of fresh fruits and vegetables increases the risk of gastric cancer. *H pylori* gastritis facilitates the growth of nitrosating bacteria, which catalyze the production of carcinogenic *N*-nitroso compounds[92]. In addition, *H pylori* infection is known to inhibit gastric secretion of ascorbic acid, which is an important scavenger of *N*-nitroso compounds and oxygen free radicals[93].

Salt-preserved foods and dietary nitrite found in preserved meats are potentially carcinogenic. Intake of salted food may increase the risk of *H pylori* infection and act synergistically to promote the development of gastric cancer. In animal models, ingestion of salt is known to cause gastritis and enhance the effects of gastric carcinogens[94,95]. Mucosal damage induced by salt may increase the possibility of persistent infection with *H pylori*[96]. Several case-control studies have shown that a high intake of salt and salt-preserved food was associated with gastric cancer risk[97-103], but evidence from prospective studies is inconsistent[104-107]. *N*-nitroso compounds are carcinogenic in animal models and are formed in the human stomach from dietary nitrite. However, case-control studies have shown a weak, nonsignificant increased risk of gastric cancer for high *vs* low nitrite intake[97,108-110]. Prospective studies have reported significant reductions in gastric cancer risk arising from fruit and vegetable consumption[111-114]. The worldwide decline in gastric cancer incidence may be attributable to the advent of refrigeration, which led to decreased consumption of preserved foods and increased intake of fresh fruits and vegetables.

Animal studies have shown that polyphenols in green tea have antitumor and anti-inflammatory effects. In preclinical studies, polyphenols have antioxidant activities and the ability to inhibit nitrosation, which have been implicated as etiologic factors of gastric cancer[115-117]. Although various case-control studies have shown a reduced risk of gastric cancer in relation to green tea consumption[118-121], recent prospective cohort studies found no protective effect of green tea on gastric cancer risk[122-125].

Table 1 Epidemiologic differences between cardia and noncardia gastric cancer

	Cardia	Noncardia
Incidence	Increasing	Decreasing
Geographic location		
Western countries	+	-
East Asia	-	+
Developing countries	-	+
Age	++	++
Male gender	++	+
Caucasian race	+	-
Low socio-economic status	-	+
H pylori infection	?	+
Diet		
Preserved foods	+	+
Fruits/vegetables	-	-
Obesity	+	?
Tobacco	+	+

NOTE: ++, strong positive association; +, positive association; -, negative association; ?, ambiguous studies.

Tobacco

Prospective studies have demonstrated a significant dose-dependent relationship between smoking and gastric cancer risk[126,127]. The effect of smoking was more pronounced for distal gastric cancer, with adjusted rate ratios of 2.0 (95% CI, 1.1-3.7) and 2.1 (95% CI, 1.2-3.6) for past and current smokers, respectively[128]. There is little support for an association between alcohol and gastric cancer[129].

Obesity

Obesity is one of the main risk factors for gastric cardia adenocarcinoma[130,131]. Obesity can promote GE reflux disease which predisposes to Barrett's esophagus, a metaplastic precursor state for adenocarcinoma of the esophagus and GE junction[132,133]. A Swedish study found that the heaviest quarter of the population had a 2.3-fold increased risk for gastric cardia adenocarcinoma compared to the lightest quartile of the population[134]. A recent prospective study from US found that body mass index was significantly associated with higher rates of stomach cancer mortality among men[135]. Thus, risk factors positively associated with adenocarcinoma of the esophagus and gastric cardia include obesity, GE reflux, and the presence of Barrett's esophagus. A summary of the main differences between cardia and noncardia gastric cancer can be found in Table 1.

Other

Less common risk factors for gastric cancer include radiation[136], pernicious anemia[137], blood type A[138], prior gastric surgery for benign conditions[139], and Epstein-Barr virus[140-142]. In addition, a positive family history is a significant risk factor, particularly with genetic syndromes such as hereditary nonpolyposis colon cancer and Li-Fraumeni syndrome[143-145].

PREVENTION OF GASTRIC CANCER

Lifestyle modifications

Because gastric cancer is often associated with a poor prognosis, the main strategy for improving clinical outcomes is through primary prevention. Reduction in gastric cancer mortality is largely due to unplanned prevention. The widespread introduction of refrigeration has led to a decrease in the intake of chemically preserved foods and increased consumption of fresh fruits and vegetables[98,146]. A decline in the prevalence of *H pylori* infection may be due to improvements in sanitary and housing conditions, as well as the use of eradication therapy[54]. In addition, reduced tobacco smoking at least in males may have contributed to the decline in gastric cancer incidence[147]. Therefore, modifiable risk factors, such as high salt and nitrite consumption, low fruit and vegetable intake, cigarette smoking, and *H pylori* infection, may be targeted for prevention.

Helicobacter pylori eradication

Public health measures to improve sanitation and housing conditions are the key factors in reducing the worldwide prevalence of *H pylori* infection. *H pylori* eradication therapy is another potential strategy for gastric cancer chemoprevention. A 7 to 14 d course of two antibiotics and an antisecretory agent has a cure rate of about 80% with durable responses[148]. However, higher reinfection rates are seen in developing countries after people have had effective eradication therapy[149]. In Japanese patients treated for early gastric cancer, *H pylori* eradication therapy resulted in a significantly lower rate of gastric cancer recurrence[150]. A randomized controlled chemoprevention trial showed that antimicrobial therapy directed against *H pylori* or dietary supplementation with antioxidants increased the regression rate of gastric atrophy and intestinal metaplasia compared to placebo[151]. In a randomized, placebo-controlled primary prevention trial conducted in a high-risk region of China, 1 630 healthy carriers of *H pylori* infection were randomized to a 2-wk course of eradication treatment or placebo[152]. Although the incidence of gastric cancer was similar in both groups after 7.5 years of follow-up, post hoc analysis of a subgroup of *H pylori* carriers without precancerous lesions at baseline showed a significant decrease in the development of gastric cancer with eradication therapy.

Several large-scale chemoprevention trials of *H pylori* eradication therapy with gastric cancer endpoints are ongoing. Potential downsides of widespread eradication therapy in asymptomatic carriers include developing antibiotic-resistant strains of *H pylori* and perhaps increasing the risk of GERD and adenocarcinoma of the esophagus and gastric cardia.

Antioxidants

High intake of antioxidants, such as vitamins C and E and β-carotene, may have a protective effect on the risk of gastric cancer. High serum levels of α-carotene, β-carotene, lycopene, and vitamin C were significantly associated with reduced risk of gastric cancer in a cohort from Shanghai, China[153]. A randomized trial in Linxian, China showed a reduced risk of both cardia and noncardia gastric cancers in individuals supplemented with a combination of selenium, β-carotene, and α-tocopherol[154]. However, a randomized trial from

Finland showed no association between α-tocopherol or β-carotene supplementation and the prevalence of gastric cancer in elderly men with atrophic gastritis[155]. Another prospective study from the US Cancer Prevention Study II cohort found that vitamin supplementation did not significantly reduce the risk of stomach cancer mortality[156]. Therefore, dietary supplementation may only play a preventive role in populations with high rates of gastric cancer and low intake of micronutrients.

COX-2 inhibitors

Cyclooxygenase-2 (COX-2) plays a role in cell proliferation, apoptosis, and angiogenesis, and may be involved in gastric carcinogenesis[157,158]. Increasing levels of COX-2 are present in the progression from atrophic gastritis to intestinal metaplasia and adenocarcinoma of the stomach[159]. Exposure to cigarette smoke, acidic conditions, and *H pylori* infection all induce COX-2 expression[160-162]. Furthermore, McCarthy *et al*. showed that COX-2 expression in the antral mucosa was reduced in the epithelium after successful eradication of *H pylori*[163].

Aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) are thought to inhibit cancer cell growth primarily through the inhibition of COX-2, and evidence is mounting that COX-2 inhibitors may be beneficial in preventing upper gastrointestinal malignancies. Compared to colorectal cancer, the association between NSAID use and the development of gastric cancer has been studied less extensively[164-166]. A recent meta-analysis showed that NSAID use was associated with a reduced risk of noncardia gastric adenocarcinoma[167]. Thus, COX-2 inhibitors may provide a chemopreventive strategy against gastric carcinogenesis.

Endoscopic screening and surveillance

Because of the high risk of gastric cancer in Japan, there has been a national endoscopic surveillance program within the commercial workforce. Annual screening with a double-contrast barium technique and endoscopy is recommended for persons over the age of 40 years[168]. With mass screening, about half of gastric tumors are being detected at an early stage in asymptomatic individuals and the mortality rate from gastric cancer has more than halved since the early 1970s[33]. An intervention study in China is underway which involves a comprehensive approach to gastric cancer prevention, including *H pylori* eradication, nutritional supplements, and aggressive screening with double contrast X-ray and endoscopic examination. In the first four years after intervention, the relative risk of overall mortality with this intervention for a high-risk group was 0.51 (95% CI, 0.35-0.74)[169]. This study suggests that targeting high-risk populations for aggressive screening and prevention may decrease gastric cancer mortality.

CONCLUSION

In summary, cardia and noncardia gastric cancers exhibit unique epidemiologic features characterized by marked geographic variation, diverging time trends, and differences based on race, sex, and socio-economic status. *H pylori* infection and dietary factors appear to be the main causative agents for distal gastric cancer, whereas GERD and obesity play a primary role in proximal gastric cancer. Future directions in primary prevention should target modifiable risk factors in high-risk populations. In the planning and evaluation of gastric cancer control activities, detailed demographic analyses will inform future screening and intervention studies.

REFERENCES

1 **Parkin DM**, Pisani P, Ferlay J. Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int J Cancer* 1993; **54**: 594-606
2 **Parkin DM**, Bray FI, Devesa SS. Cancer burden in the year 2000. The global picture. *Eur J Cancer* 2001; **37 Suppl 8**: S4-66
3 **Parkin DM**. International variation. *Oncogene* 2004; **23**: 6329-6340
4 **Stewart BW**, Kleihues P. World Cancer Report. Lyon: IARC Press, 2003
5 **Blot WJ**, Devesa SS, Kneller RW, Fraumeni JF. Rising incidence of adenocarcinoma of the esophagus and gastric cardia. *JAMA* 1991; **265**: 1287-1289
6 **Brown LM**, Devesa SS. Epidemiologic trends in esophageal and gastric cancer in the United States. *Surg Oncol Clin N Am* 2002; **11**: 235-256
7 **Lauren P.** The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49
8 **Correa P**, Sasano N, Stemmermann GN, Haenszel W. Pathology of gastric carcinoma in Japanese populations: comparisons between Miyagi prefecture, Japan, and Hawaii. *J Natl Cancer Inst* 1973; **51**: 1449-1459
9 **Munoz N**. Gastric Carcinogenesis. In: Reed PI, Hill MJ, eds. Gastric carcinogenesis: proceedings of the 6th Annual Symposium of the European Organization for Cooperation in Cancer Prevention Studies (ECP). Amsterdam: Elsevier Science, 1988: 51-69
10 **Muñoz N**, Correa P, Cuello C, Duque E. Histologic types of gastric carcinoma in high- and low-risk areas. *Int J Cancer* 1968; **3**: 809-818
11 **Kaneko S**, Yoshimura T. Time trend analysis of gastric cancer incidence in Japan by histological types, 1975-1989. *Br J Cancer* 2001; **84**: 400-405
12 **Henson DE**, Dittus C, Younes M, Nguyen H, Albores-Saavedra J. Differential trends in the intestinal and diffuse types of gastric carcinoma in the United States, 1973-2000: increase in the signet ring cell type. *Arch Pathol Lab Med* 2004; **128**: 765-770
13 **Pera M**, Cameron AJ, Trastek VF, Carpenter HA, Zinsmeister AR. Increasing incidence of adenocarcinoma of the esophagus and esophagogastric junction. *Gastroenterology* 1993; **104**: 510-513
14 **Hansen S**, Wiig JN, Giercksky KE, Tretli S. Esophageal and gastric carcinoma in Norway 1958-1992: incidence time trend variability according to morphological subtypes and organ subsites. *Int J Cancer* 1997; **71**: 340-344
15 **Møller H**. Incidence of cancer of oesophagus, cardia and stomach in Denmark. *Eur J Cancer Prev* 1992; **1**: 159-164
16 **Harrison SL**, Goldacre MJ, Seagroatt V. Trends in registered incidence of oesophageal and stomach cancer in the Oxford region, 1974-88. *Eur J Cancer Prev* 1992; **1**: 271-274
17 **Levi F**, La Vecchia C, Te VC. Descriptive epidemiology of adenocarcinomas of the cardia and distal stomach in the Swiss Canton of Vaud. *Tumori* 1990; **76**: 167-171
18 **Armstrong RW**, Borman B. Trends in incidence rates of adenocarcinoma of the oesophagus and gastric cardia in New Zealand, 1978-1992. *Int J Epidemiol* 1996; **25**: 941-947
19 **Thomas RJ**, Lade S, Giles GG, Thursfield V. Incidence trends in oesophageal and proximal gastric carcinoma in Victoria. *Aust N Z J Surg* 1996; **66**: 271-275

20 **Parkin DM**, Whelan SL, Ferlay J. Cancer Incidence in Five Continents. Vol VII. Lyon, France: International Agency for Research on Cancer, 1997: 822-823
21 **Yamamoto S**. Stomach cancer incidence in the world. *Jpn J Clin Oncol* 2001; **31**: 471
22 **Ahn YO**, Park BJ, Yoo KY, Kim NK, Heo DS, Lee JK, Ahn HS, Kang DH, Kim H, Lee MS. Incidence estimation of stomach cancer among Koreans. *J Korean Med Sci* 1991; **6**: 7-14
23 **Nomura A**. Stomach Cancer. In: Schottenfeld D, Fraumeni JF, eds. Cancer Epidemiology and Prevention. 2nd ed. New York: Oxford University Press, 1996: 707-724
24 **Liu Y**, Kaneko S, Sobue T. Trends in reported incidences of gastric cancer by tumour location, from 1975 to 1989 in Japan. *Int J Epidemiol* 2004; **33**: 808-815
25 **McMichael AJ**, McCall MG, Hartshorne JM, Woodings TL. Patterns of gastro-intestinal cancer in European migrants to Australia: the role of dietary change. *Int J Cancer* 1980; **25**: 431-437
26 **El-Serag HB**, Mason AC, Petersen N, Key CR. Epidemiological differences between adenocarcinoma of the oesophagus and adenocarcinoma of the gastric cardia in the USA. *Gut* 2002; **50**: 368-372
27 **Powell J**, McConkey CC. The rising trend in oesophageal adenocarcinoma and gastric cardia. *Eur J Cancer Prev* 1992; **1**: 265-269
28 **Shen H**, Xu Y, Qian Y, Yu R, Qin Y, Zhou L, Wang X, Spitz MR, Wei Q. Polymorphisms of the DNA repair gene XRCC1 and risk of gastric cancer in a Chinese population. *Int J Cancer* 2000; **88**: 601-606
29 **Jemal A**, Thomas A, Murray T, Thun M. Cancer statistics, 2002. *CA Cancer J Clin* 202; **52**: 23-47
30 **Ries LA**, Kosary CL, Hankey BF. SEER Cancer Statistics Review 1973-1995. Bethesda: U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, National Cancer Institute, 1998
31 **Verdecchia A**, Corazziari I, Gatta G, Lisi D, Faivre J, Forman D. Explaining gastric cancer survival differences among European countries. *Int J Cancer* 2004; **109**: 737-741
32 **Fielding JWL**, Powell J, Allum WH. Cancer of the Stomach. London: The Macmillan Press, 1989
33 IARC Unit of Descriptive Epidemiology: WHO cancer mortality databank. Cancer Mondial, 2001. Available from: URL: http://www-dep.iarc.fr/ataava/globocan/who.htm
34 **Ries LAG**, Kosary CL, Hankey BF, Miller BA, Harras A, Edwards BK. SEER Cancer Statistics Review 1973-1994, National Cancer Institute, NIH Publication No. 97-2789. Bethesda: Department of Health and Human Services, 1997
35 **Faivre J**, Forman D, Estève J, Gatta G. Survival of patients with oesophageal and gastric cancers in Europe. EUROCARE Working Group. *Eur J Cancer* 1998; **34**: 2167-2175
36 Berrino F, Capocaccia R, Esteve J. Survival of Cancer Patients in Europe: The EUROCARE-2 Study. IARC Scientific Publications No. 151. Lyon: IARC, 1999
37 **Theuer CP**, Kurosaki T, Ziogas A, Butler J, Anton-Culver H. Asian patients with gastric carcinoma in the United States exhibit unique clinical features and superior overall and cancer specific survival rates. *Cancer* 2000; **89**: 1883-1892
38 **Marshall BJ**, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; **1**: 1311-1315
39 **Parsonnet J**. The incidence of Helicobacter pylori infection. *Aliment Pharmacol Ther* 1995; **9 Suppl 2**: 45-51
40 **Howson CP**, Hiyama T, Wynder EL. The decline in gastric cancer: epidemiology of an unplanned triumph. *Epidemiol Rev* 1986; **8**: 1-27
41 **Dooley CP**, Cohen H, Fitzgibbons PL, Bauer M, Appleman MD, Perez-Perez GI, Blaser MJ. Prevalence of Helicobacter pylori infection and histologic gastritis in asymptomatic persons. *N Engl J Med* 1989; **321**: 1562-1566
42 **Asaka M**, Kimura T, Kudo M, Takeda H, Mitani S, Miyazaki T, Miki K, Graham DY. Relationship of Helicobacter pylori to serum pepsinogens in an asymptomatic Japanese population. *Gastroenterology* 1992; **102**: 760-766
43 **Youn HS**, Ko GH, Chung MH, Lee WK, Cho MJ, Rhee KH. Pathogenesis and prevention of stomach cancer. *J Korean Med Sci* 1996; **11**: 373-385
44 **Feldman RA.** Epidemiologic observations and open questions about disease and infection caused by Helicobacter pylori. In: Achtman M, Serbaum S. Helicobacter pylori: Molecular and Cellular Biology. Wymondham: Horizon Scientific, 2001: 29-51
45 **Buckley MJ**, O'Shea J, Grace A, English L, Keane C, Hourihan D, O'Morain CA. A community-based study of the epidemiology of Helicobacter pylori infection and associated asymptomatic gastroduodenal pathology. *Eur J Gastroenterol Hepatol* 1998; **10**: 375-379
46 **Webb PM**, Knight T, Greaves S, Wilson A, Newell DG, Elder J, Forman D. Relation between infection with Helicobacter pylori and living conditions in childhood: evidence for person to person transmission in early life. *BMJ* 1994; **308**: 750-753
47 **Kurosawa M**, Kikuchi S, Inaba Y, Ishibashi T, Kobayashi F. Helicobacter pylori infection among Japanese children. *J Gastroenterol Hepatol* 2000; **15**: 1382-1385
48 **Olmos JA**, Ríos H, Higa R. Prevalence of Helicobacter pylori infection in Argentina: results of a nationwide epidemiologic study. Argentinean Hp Epidemiologic Study Group. *J Clin Gastroenterol* 2000; **31**: 33-37
49 **Goodman KJ**, Correa P. Transmission of Helicobacter pylori among siblings. *Lancet* 2000; **355**: 358-362
50 **Parsonnet J**, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131
51 **Forman D**, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sitas F. Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. *BMJ* 1991; **302**: 1302-1305
52 **Nomura A**, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med* 1991; **325**: 1132-1136
53 An international association between Helicobacter pylori infection and gastric cancer. The EUROGAST Study Group. *Lancet* 1993; **341**: 1359-1362
54 International Agency for Research on Cancer Working Group on the Evaluation of Carcinogenic Risks to Humans. Schistosomes, Liver Flukes, and Helicobacter pylori. Lyon: International Agency for Research on Cancer, 1994: 177-240
55 **Correa P**. Helicobacter pylori and gastric cancer: state of the art. *Cancer Epidemiol Biomarkers Prev* 1996; **5**: 477-481
56 **Sipponen P**, Kosunen TU, Valle J, Riihelä M, Seppälä K. Helicobacter pylori infection and chronic gastritis in gastric cancer. *J Clin Pathol* 1992; **45**: 319-323
57 **Hansson LE**, Engstrand L, Nyrén O, Evans DJ, Lindgren A, Bergström R, Andersson B, Athlin L, Bendtsen O, Tracz P. Helicobacter pylori infection: independent risk indicator of gastric adenocarcinoma. *Gastroenterology* 1993; **105**: 1098-1103
58 **Hu PJ**, Mitchell HM, Li YY, Zhou MH, Hazell SL. Association of Helicobacter pylori with gastric cancer and observations on the detection of this bacterium in gastric cancer cases. *Am J Gastroenterol* 1994; **89**: 1806-1810
59 **Kikuchi S**, Wada O, Nakajima T, Nishi T, Kobayashi O, Konishi T, Inaba Y. Serum anti-Helicobacter pylori antibody and gastric carcinoma among young adults. Research Group on Prevention of Gastric Carcinoma among Young Adults. *Cancer* 1995; **75**: 2789-2793
60 **Kokkola A**, Valle J, Haapiainen R, Sipponen P, Kivilaakso E, Puolakkainen P. Helicobacter pylori infection in young patients with gastric carcinoma. *Scand J Gastroenterol* 1996; **31**: 643-647
61 **Barreto-Zuñiga R**, Maruyama M, Kato Y, Aizu K, Ohta H, Takekoshi T, Bernal SF. Significance of Helicobacter pylori infection as a risk factor in gastric cancer: serological and histological studies. *J Gastroenterol* 1997; **32**: 289-294
62 **Miehlke S**, Hackelsberger A, Meining A, von Arnim U, Müller P, Ochsenkühn T, Lehn N, Malfertheiner P, Stolte M, Bayerdörffer E. Histological diagnosis of Helicobacter pylori gastritis is predictive of a high risk of gastric carcinoma. *Int J Cancer* 1997;

73: 837-839
63 **Nomura AM**, Stemmermann GN, Chyou PH. Gastric cancer among the Japanese in Hawaii. *Jpn J Cancer Res* 1995; **86**: 916-923
64 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789
65 **Tomb JF**, White O, Kerlavage AR, Clayton RA, Sutton GG, Fleischmann RD, Ketchum KA, Klenk HP, Gill S, Dougherty BA, Nelson K, Quackenbush J, Zhou L, Kirkness EF, Peterson S, Loftus B, Richardson D, Dodson R, Khalak HG, Glodek A, McKenney K, Fitzegerald LM, Lee N, Adams MD, Hickey EK, Berg DE, Gocayne JD, Utterback TR, Peterson JD, Kelley JM, Cotton MD, Weidman JM, Fujii C, Bowman C, Watthey L, Wallin E, Hayes WS, Borodovsky M, Karp PD, Smith HO, Fraser CM, Venter JC. The complete genome sequence of the gastric pathogen Helicobacter pylori. *Nature* 1997; **388**: 539-547
66 **Alm RA**, Ling LS, Moir DT, King BL, Brown ED, Doig PC, Smith DR, Noonan B, Guild BC, deJonge BL, Carmel G, Tummino PJ, Caruso A, Uria-Nickelsen M, Mills DM, Ives C, Gibson R, Merberg D, Mills SD, Jiang Q, Taylor DE, Vovis GF, Trust TJ. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori. *Nature* 1999; **397**: 176-180
67 **Kuipers EJ**, Pérez-Pérez GI, Meuwissen SG, Blaser MJ. Helicobacter pylori and atrophic gastritis: importance of the cagA status. *J Natl Cancer Inst* 1995; **87**: 1777-1780
68 **Blaser MJ**, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995; **55**: 2111-2115
69 **Parsonnet J**, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. *Gut* 1997; **40**: 297-301
70 **Huang JQ**, Zheng GF, Sumanac K, Irvine EJ, Hunt RH. Meta-analysis of the relationship between cagA seropositivity and gastric cancer. *Gastroenterology* 2003; **125**: 1636-1644
71 **Vicari JJ**, Peek RM, Falk GW, Goldblum JR, Easley KA, Schnell J, Perez-Perez GI, Halter SA, Rice TW, Blaser MJ, Richter JE. The seroprevalence of cagA-positive Helicobacter pylori strains in the spectrum of gastroesophageal reflux disease. *Gastroenterology* 1998; **115**: 50-57
72 **Ito Y**, Azuma T, Ito S, Miyaji H, Hirai M, Yamazaki Y, Sato F, Kato T, Kohli Y, Kuriyama M. Analysis and typing of the vacA gene from cagA-positive strains of Helicobacter pylori isolated in Japan. *J Clin Microbiol* 1997; **35**: 1710-1714
73 **Azuma T**, Yamakawa A, Yamazaki S, Fukuta K, Ohtani M, Ito Y, Dojo M, Yamazaki Y, Kuriyama M. Correlation between variation of the 3′ region of the cagA gene in Helicobacter pylori and disease outcome in Japan. *J Infect Dis* 2002; **186**: 1621-1630
74 **El-Omar EM**, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF, Rabkin CS. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; **404**: 398-402
75 **El-Omar EM**, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, Fraumeni JF, Chow WH. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology* 2003; **124**: 1193-1201
76 **Blaser MJ**. Hypothesis: the changing relationships of Helicobacter pylori and humans: implications for health and disease. *J Infect Dis* 1999; **179**: 1523-1530
77 **Helicobacter and Cancer Collaborative Group.** Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts. *Gut* 2001; **49**: 347-353
78 **Hansen S**, Melby KK, Aase S, Jellum E, Vollset SE. Helicobacter pylori infection and risk of cardia cancer and non-cardia gastric cancer. A nested case-control study. *Scand J Gastroenterol* 1999; **34**: 353-360
79 **Chow WH**, Blaser MJ, Blot WJ, Gammon MD, Vaughan TL, Risch HA, Perez-Perez GI, Schoenberg JB, Stanford JL, Rotterdam H, West AB, Fraumeni JF. An inverse relation between cagA+ strains of Helicobacter pylori infection and risk of esophageal and gastric cardia adenocarcinoma. *Cancer Res* 1998; **58**: 588-590
80 **Warburton-Timms VJ**, Charlett A, Valori RM, Uff JS, Shepherd NA, Barr H, McNulty CA. The significance of cagA(+) Helicobacter pylori in reflux oesophagitis. *Gut* 2001; **49**: 341-346
81 **El-Serag HB**, Sonnenberg A, Jamal MM, Inadomi JM, Crooks LA, Feddersen RM. Corpus gastritis is protective against reflux oesophagitis. *Gut* 1999; **45**: 181-185
82 **Koike T**, Ohara S, Sekine H, Iijima K, Abe Y, Kato K, Toyota T, Shimosegawa T. Helicobacter pylori infection prevents erosive reflux oesophagitis by decreasing gastric acid secretion. *Gut* 2001; **49**: 330-334
83 **Raghunath A**, Hungin AP, Wooff D, Childs S. Prevalence of Helicobacter pylori in patients with gastro-oesophageal reflux disease: systematic review. *BMJ* 2003; **326**: 737
84 **Labenz J**, Malfertheiner P. Helicobacter pylori in gastro-oesophageal reflux disease: causal agent, independent or protective factor? *Gut* 1997; **41**: 277-280
85 **Bytzer P**, Aalykke C, Rune S, Weywadt L, Gjørup T, Eriksen J, Bonnevie O, Bekker C, Kromann-Andersen H, Kjaergaard J, Rask-Madsen J, Vilien M, Hansen J, Justesen T, Vyberg M, Teglbjaerg PS. Eradication of Helicobacter pylori compared with long-term acid suppression in duodenal ulcer disease. A randomized trial with 2-year follow-up. The Danish Ulcer Study Group. *Scand J Gastroenterol* 2000; **35**: 1023-1032
86 **Vakil N**, Hahn B, McSorley D. Recurrent symptoms and gastro-oesophageal reflux disease in patients with duodenal ulcer treated for Helicobacter pylori infection. *Aliment Pharmacol Ther* 2000; **14**: 45-51
87 **McColl KE**, Dickson A, El-Nujumi A, El-Omar E, Kelman A. Symptomatic benefit 1-3 years after H. pylori eradication in ulcer patients: impact of gastroesophageal reflux disease. *Am J Gastroenterol* 2000; **95**: 101-105
88 **Manes G**, Mosca S, De Nucci C, Lombardi G, Lioniello M, Balzano A. High prevalence of reflux symptoms in duodenal ulcer patients who develop gastro-oesophageal reflux disease after curing Helicobacter pylori infection. *Dig Liver Dis* 2001; **33**: 665-670
89 **Befrits R**, Sjöstedt S, Odman B, Sörngård H, Lindberg G. Curing Helicobacter pylori infection in patients with duodenal ulcer does not provoke gastroesophageal reflux disease. *Helicobacter* 2000; **5**: 202-205
90 **Sasaki A**, Haruma K, Manabe N, Tanaka S, Yoshihara M, Chayama K. Long-term observation of reflux oesophagitis developing after Helicobacter pylori eradication therapy. *Aliment Pharmacol Ther* 2003; **17**: 1529-1534
91 **Laine L**, Sugg J. Effect of Helicobacter pylori eradication on development of erosive esophagitis and gastroesophageal reflux disease symptoms: a post hoc analysis of eight double blind prospective studies. *Am J Gastroenterol* 2002; **97**: 2992-2997
92 **Sanduleanu S**, Jonkers D, De Bruine A, Hameeteman W, Stockbrügger RW. Non-Helicobacter pylori bacterial flora during acid-suppressive therapy: differential findings in gastric juice and gastric mucosa. *Aliment Pharmacol Ther* 2001; **15**: 379-388
93 **O'Connor HJ**, Schorah CJ, Habibzedah N, Axon AT, Cockel R. Vitamin C in the human stomach: relation to gastric pH, gastroduodenal disease, and possible sources. *Gut* 1989; **30**: 436-442
94 **Tatematsu M**, Takahashi M, Fukushima S, Hananouchi M, Shirai T. Effects in rats of sodium chloride on experimental gastric cancers induced by N-methyl-N-nitro-N-nitrosoguanidine or 4-nitroquinoline-1-oxide. *J Natl Cancer Inst* 1975; **55**: 101-106
95 **Takahashi M**, Hasegawa R. Enhancing effects of dietary salt on both initiation and promotion stages of rat gastric carcinogenesis. *Princess Takamatsu Symp* 1985; **16**: 169-182
96 **Fox JG**, Dangler CA, Taylor NS, King A, Koh TJ, Wang TC. High-salt diet induces gastric epithelial hyperplasia and parietal cell loss, and enhances Helicobacter pylori colonization in C57BL/6 mice. *Cancer Res* 1999; **59**: 4823-4828
97 **Kono S**, Hirohata T. Nutrition and stomach cancer. *Cancer*

Causes Control 1996; **7**: 41-55
98 World Cancer Research Fund, American Institute for Cancer Research. Food, Nutrition and the Prevention of Cancer: a Global Perspective. Washington, D.C.: American Institute for Cancer Research, 1997
99 **Ward MH**, López-Carrillo L. Dietary factors and the risk of gastric cancer in Mexico City. *Am J Epidemiol* 1999; **149**: 925-932
100 **Kim HJ**, Chang WK, Kim MK, Lee SS, Choi BY. Dietary factors and gastric cancer in Korea: a case-control study. *Int J Cancer* 2002; **97**: 531-535
101 **Lee SA**, Kang D, Shim KN, Choe JW, Hong WS, Choi H. Effect of diet and Helicobacter pylori infection to the risk of early gastric cancer. *J Epidemiol* 2003; **13**: 162-168
102 **Joossens JV**, Hill MJ, Elliott P, Stamler R, Lesaffre E, Dyer A, Nichols R, Kesteloot H. Dietary salt, nitrate and stomach cancer mortality in 24 countries. European Cancer Prevention (ECP) and the INTERSALT Cooperative Research Group. *Int J Epidemiol* 1996; **25**: 494-504
103 **Tsugane S**, Tsuda M, Gey F, Watanabe S. Cross-sectional study with multiple measurements of biological markers for assessing stomach cancer risks at the population level. *Environ Health Perspect* 1992; **98**: 207-210
104 **Kato I**, Tominaga S, Matsumoto K. A prospective study of stomach cancer among a rural Japanese population: a 6-year survey. *Jpn J Cancer Res* 1992; **83**: 568-575
105 **Kneller RW**, McLaughlin JK, Bjelke E, Schuman LM, Blot WJ, Wacholder S, Gridley G, CoChien HT, Fraumeni JF. A cohort study of stomach cancer in a high-risk American population. *Cancer* 1991; **68**: 672-678
106 **Nomura A**, Grove JS, Stemmermann GN, Severson RK. A prospective study of stomach cancer and its relation to diet, cigarettes, and alcohol consumption. *Cancer Res* 1990; **50**: 627-631
107 **Tsugane S**, Sasazuki S, Kobayashi M, Sasaki S. Salt and salted food intake and subsequent risk of gastric cancer among middle-aged Japanese men and women. *Br J Cancer* 2004; **90**: 128-134
108 **Buiatti E**, Palli D, Decarli A, Amadori D, Avellini C, Bianchi S, Bonaguri C, Cipriani F, Cocco P, Giacosa A. A case-control study of gastric cancer and diet in Italy: II. Association with nutrients. *Int J Cancer* 1990; **45**: 896-901
109 **La Vecchia C**, Ferraroni M, D'Avanzo B, Decarli A, Franceschi S. Selected micronutrient intake and the risk of gastric cancer. *Cancer Epidemiol Biomarkers Prev* 1994; **3**: 393-398
110 **Hansson LE**, Nyrén O, Bergström R, Wolk A, Lindgren A, Baron J, Adami HO. Nutrients and gastric cancer risk. A population-based case-control study in Sweden. *Int J Cancer* 1994; **57**: 638-644
111 **McCullough ML**, Robertson AS, Jacobs EJ, Chao A, Calle EE, Thun MJ. A prospective study of diet and stomach cancer mortality in United States men and women. *Cancer Epidemiol Biomarkers Prev* 2001; **10**: 1201-1205
112 **Hirayama T**. A large scale cohort study on cancer risk by diet-with special reference to the risk reducing effects of green-yellow vegetable consumption. In: Hayashi Y, Nagao M, Sugimura T. Diet, nutrition and cancer: proceedings of the 16th International Symposium of the Princess Takamatsu Cancer Research Fund. Utrecht: Tokyo/VNU Science, 1986: 41-53
113 **Hertog MG**, Bueno-de-Mesquita HB, Fehily AM, Sweetnam PM, Elwood PC, Kromhout D. Fruit and vegetable consumption and cancer mortality in the Caerphilly Study. *Cancer Epidemiol Biomarkers Prev* 1996; **5**: 673-677
114 **Kobayashi M**, Tsubono Y, Sasazuki S, Sasaki S, Tsugane S. Vegetables, fruit and risk of gastric cancer in Japan: a 10-year follow-up of the JPHC Study Cohort I. *Int J Cancer* 2002; **102**: 39-44
115 **Wang ZY**, Cheng SJ, Zhou ZC, Athar M, Khan WA, Bickers DR, Mukhtar H. Antimutagenic activity of green tea polyphenols. *Mutat Res* 1989; **223**: 273-285
116 **Wang ZY**, Hong JY, Huang MT, Reuhl KR, Conney AH, Yang CS. Inhibition of N-nitrosodiethylamine- and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced tumorigenesis in A/J mice by green tea and black tea. *Cancer Res* 1992; **52**: 1943-1947
117 **Xu Y**, Ho CT, Amin SG, Han C, Chung FL. Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants. *Cancer Res* 1992; **52**: 3875-3879
118 **Setiawan VW**, Zhang ZF, Yu GP, Lu QY, Li YL, Lu ML, Wang MR, Guo CH, Yu SZ, Kurtz RC, Hsieh CC. Protective effect of green tea on the risks of chronic gastritis and stomach cancer. *Int J Cancer* 2001; **92**: 600-604
119 **Yu GP**, Hsieh CC, Wang LY, Yu SZ, Li XL, Jin TH. Green-tea consumption and risk of stomach cancer: a population-based case-control study in Shanghai, China. *Cancer Causes Control* 1995; **6**: 532-538
120 **Ji BT**, Chow WH, Yang G, McLaughlin JK, Gao RN, Zheng W, Shu XO, Jin F, Fraumeni JF, Gao YT. The influence of cigarette smoking, alcohol, and green tea consumption on the risk of carcinoma of the cardia and distal stomach in Shanghai, China. *Cancer* 1996; **77**: 2449-2457
121 **Inoue M**, Tajima K, Hirose K, Hamajima N, Takezaki T, Kuroishi T, Tominaga S. Tea and coffee consumption and the risk of digestive tract cancers: data from a comparative case-referent study in Japan. *Cancer Causes Control* 1998; **9**: 209-216
122 **Tsubono Y**, Nishino Y, Komatsu S, Hsieh CC, Kanemura S, Tsuji I, Nakatsuka H, Fukao A, Satoh H, Hisamichi S. Green tea and the risk of gastric cancer in Japan. *N Engl J Med* 2001; **344**: 632-636
123 **Hoshiyama Y**, Kawaguchi T, Miura Y, Mizoue T, Tokui N, Yatsuya H, Sakata K, Kondo T, Kikuchi S, Toyoshima H, Hayakawa N, Tamakoshi A, Ohno Y, Yoshimura T. A prospective study of stomach cancer death in relation to green tea consumption in Japan. *Br J Cancer* 2002; **87**: 309-313
124 **Nagano J**, Kono S, Preston DL, Mabuchi K. A prospective study of green tea consumption and cancer incidence, Hiroshima and Nagasaki (Japan). *Cancer Causes Control* 2001; **12**: 501-508
125 **Galanis DJ**, Kolonel LN, Lee J, Nomura A. Intakes of selected foods and beverages and the incidence of gastric cancer among the Japanese residents of Hawaii: a prospective study. *Int J Epidemiol* 1998; **27**: 173-180
126 **Koizumi Y**, Tsubono Y, Nakaya N, Kuriyama S, Shibuya D, Matsuoka H, Tsuji I. Cigarette smoking and the risk of gastric cancer: a pooled analysis of two prospective studies in Japan. *Int J Cancer* 2004; **112**: 1049-1055
127 **González CA**, Pera G, Agudo A, Palli D, Krogh V, Vineis P, Tumino R, Panico S, Berglund G, Simán H, Nyrén O, Agren A, Martinez C, Dorronsoro M, Barricarte A, Tormo MJ, Quiros JR, Allen N, Bingham S, Day N, Miller A, Nagel G, Boeing H, Overvad K, Tjonneland A, Bueno-De-Mesquita HB, Boshuizen HC, Peeters P, Numans M, Clavel-Chapelon F, Helen I, Agapitos E, Lund E, Fahey M, Saracci R, Kaaks R, Riboli E. Smoking and the risk of gastric cancer in the European Prospective Investigation Into Cancer and Nutrition (EPIC). *Int J Cancer* 2003; **107**: 629-634
128 **Chao A**, Thun MJ, Henley SJ, Jacobs EJ, McCullough ML, Calle EE. Cigarette smoking, use of other tobacco products and stomach cancer mortality in US adults: The Cancer Prevention Study II. *Int J Cancer* 2002; **101**: 380-389
129 **Franceschi S**, La Vecchia C. Alcohol and the risk of cancers of the stomach and colon-rectum. *Dig Dis* 1994; **12**: 276-289
130 **Chow WH**, Blot WJ, Vaughan TL, Risch HA, Gammon MD, Stanford JL, Dubrow R, Schoenberg JB, Mayne ST, Farrow DC, Ahsan H, West AB, Rotterdam H, Niwa S, Fraumeni JF. Body mass index and risk of adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst* 1998; **90**: 150-155
131 **Vaughan TL**, Davis S, Kristal A, Thomas DB. Obesity, alcohol, and tobacco as risk factors for cancers of the esophagus and gastric cardia: adenocarcinoma versus squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev* 1995; **4**: 85-92
132 **Clark GW**, Smyrk TC, Burdiles P, Hoeft SF, Peters JH, Kiyabu M, Hinder RA, Bremner CG, DeMeester TR. Is Barrett's metaplasia the source of adenocarcinomas of the cardia? *Arch Surg* 1994; **129**: 609-614
133 **Ishaq S**, Jankowski JA. Barrett's metaplasia: clinical implications. *World J Gastroenterol* 2001; **7**: 563-565

134 **Lagergren J**, Bergström R, Nyrén O. Association between body mass and adenocarcinoma of the esophagus and gastric cardia. *Ann Intern Med* 1999; **130**: 883-890

135 **Calle EE**, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003; **348**: 1625-1638

136 **Thompson DE**, Mabuchi K, Ron E, Soda M, Tokunaga M, Ochikubo S, Sugimoto S, Ikeda T, Terasaki M, Izumi S. Cancer incidence in atomic bomb survivors. Part II: Solid tumors, 1958-1987. *Radiat Res* 1994; **137**: S17-S67

137 **Hsing AW**, Hansson LE, McLaughlin JK, Nyren O, Blot WJ, Ekbom A, Fraumeni JF. Pernicious anemia and subsequent cancer. A population-based cohort study. *Cancer* 1993; **71**: 745-750

138 **Aird I**, Bentall HH, Roberts JA. A relationship between cancer of stomach and the ABO blood groups. *Br Med J* 1953; **1**: 799-801

139 **Stalnikowicz R**, Benbassat J. Risk of gastric cancer after gastric surgery for benign disorders. *Arch Intern Med* 1990; **150**: 2022-2026

140 **Levine PH**, Stemmermann G, Lennette ET, Hildesheim A, Shibata D, Nomura A. Elevated antibody titers to Epstein-Barr virus prior to the diagnosis of Epstein-Barr-virus-associated gastric adenocarcinoma. *Int J Cancer* 1995; **60**: 642-644

141 **Uemura Y**, Tokunaga M, Arikawa J, Yamamoto N, Hamasaki Y, Tanaka S, Sato E, Land CE. A unique morphology of Epstein-Barr virus-related early gastric carcinoma. *Cancer Epidemiol Biomarkers Prev* 1994; **3**: 607-611

142 **Shousha S**, Luqmani YA. Epstein-Barr virus in gastric carcinoma and adjacent normal gastric and duodenal mucosa. *J Clin Pathol* 1994; **47**: 695-698

143 **Palli D**, Galli M, Caporaso NE, Cipriani F, Decarli A, Saieva C, Fraumeni JF, Buiatti E. Family history and risk of stomach cancer in Italy. *Cancer Epidemiol Biomarkers Prev* 1994; **3**: 15-18

144 **La Vecchia C**, Negri E, Franceschi S, Gentile A. Family history and the risk of stomach and colorectal cancer. *Cancer* 1992; **70**: 50-55

145 **Lissowska J**, Groves FD, Sobin LH, Fraumeni JF, Nasierowska-Guttmejer A, Radziszewski J, Regula J, Hsing AW, Zatonski W, Blot WJ, Chow WH. Family history and risk of stomach cancer in Warsaw, Poland. *Eur J Cancer Prev* 1999; **8**: 223-227

146 **La Vecchia C**, Negri E, D'Avanzo B, Franceschi S. Electric refrigerator use and gastric cancer risk. *Br J Cancer* 1990; **62**: 136-137

147 **Trédaniel J**, Boffetta P, Buiatti E, Saracci R, Hirsch A. Tobacco smoking and gastric cancer: review and meta-analysis. *Int J Cancer* 1997; **72**: 565-573

148 **Suerbaum S**, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; **347**: 1175-1186

149 **Coelho LG**, Passos MC, Chausson Y, Costa EL, Maia AF, Brandao MJ, Rodrigues DC, Castro LP. Duodenal ulcer and eradication of Helicobacter pylori in a developing country. An 18-month follow-up study. *Scand J Gastroenterol* 1992; **27**: 362-366

150 **Uemura N**, Mukai T, Okamoto S, Yamaguchi S, Mashiba H, Taniyama K, Sasaki N, Haruma K, Sumii K, Kajiyama G. Effect of Helicobacter pylori eradication on subsequent development of cancer after endoscopic resection of early gastric cancer. *Cancer Epidemiol Biomarkers Prev* 1997; **6**: 639-642

151 **Correa P**, Fontham ET, Bravo JC, Bravo LE, Ruiz B, Zarama G, Realpe JL, Malcom GT, Li D, Johnson WD, Mera R. Chemoprevention of gastric dysplasia: randomized trial of antioxidant supplements and anti-helicobacter pylori therapy. *J Natl Cancer Inst* 2000; **92**: 1881-1888

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

153 **Yuan JM**, Ross RK, Gao YT, Qu YH, Chu XD, Yu MC. Prediagnostic levels of serum micronutrients in relation to risk of gastric cancer in Shanghai, China. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 1772-1780

154 **Blot WJ**, Li JY, Taylor PR, Guo W, Dawsey S, Wang GQ, Yang CS, Zheng SF, Gail M, Li GY. Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst* 1993; **85**: 1483-1492

155 **Varis K**, Taylor PR, Sipponen P, Samloff IM, Heinonen OP, Albanes D, Härkönen M, Huttunen JK, Laxén F, Virtamo J. Gastric cancer and premalignant lesions in atrophic gastritis: a controlled trial on the effect of supplementation with alpha-tocopherol and beta-carotene. The Helsinki Gastritis Study Group. *Scand J Gastroenterol* 1998; **33**: 294-300

156 **Jacobs EJ**, Connell CJ, McCullough ML, Chao A, Jonas CR, Rodriguez C, Calle EE, Thun MJ. Vitamin C, vitamin E, and multivitamin supplement use and stomach cancer mortality in the Cancer Prevention Study II cohort. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 35-41

157 **Wong BC**, Zhu GH, Lam SK. Aspirin induced apoptosis in gastric cancer cells. *Biomed Pharmacother* 1999; **53**: 315-318

158 **Sawaoka H**, Tsuji S, Tsujii M, Gunawan ES, Sasaki Y, Kawano S, Hori M. Cyclooxygenase inhibitors suppress angiogenesis and reduce tumor growth in vivo. *Lab Invest* 1999; **79**: 1469-1477

159 **Ristimäki A**, Honkanen N, Jänkälä H, Sipponen P, Härkönen M. Expression of cyclooxygenase-2 in human gastric carcinoma. *Cancer Res* 1997; **57**: 1276-1280

160 **Kelley DJ**, Mestre JR, Subbaramaiah K, Sacks PG, Schantz SP, Tanabe T, Inoue H, Ramonetti JT, Dannenberg AJ. Benzo[a]pyrene up-regulates cyclooxygenase-2 gene expression in oral epithelial cells. *Carcinogenesis* 1997; **18**: 795-799

161 **Shirvani VN**, Ouatu-Lascar R, Kaur BS, Omary MB, Triadafilopoulos G. Cyclooxygenase 2 expression in Barrett's esophagus and adenocarcinoma: Ex vivo induction by bile salts and acid exposure. *Gastroenterology* 2000; **118**: 487-496

162 **Fu S**, Ramanujam KS, Wong A, Fantry GT, Drachenberg CB, James SP, Meltzer SJ, Wilson KT. Increased expression and cellular localization of inducible nitric oxide synthase and cyclooxygenase 2 in Helicobacter pylori gastritis. *Gastroenterology* 1999; **116**: 1319-1329

163 **McCarthy CJ**, Crofford LJ, Greenson J, Scheiman JM. Cyclooxygenase-2 expression in gastric antral mucosa before and after eradication of Helicobacter pylori infection. *Am J Gastroenterol* 1999; **94**: 1218-1223

164 **Farrow DC**, Vaughan TL, Hansten PD, Stanford JL, Risch HA, Gammon MD, Chow WH, Dubrow R, Ahsan H, Mayne ST, Schoenberg JB, West AB, Rotterdam H, Fraumeni JF, Blot WJ. Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. *Cancer Epidemiol Biomarkers Prev* 1998; **7**: 97-102

165 **Akre K**, Ekström AM, Signorello LB, Hansson LE, Nyrén O. Aspirin and risk for gastric cancer: a population-based case-control study in Sweden. *Br J Cancer* 2001; **84**: 965-968

166 **Coogan PF**, Rosenberg L, Palmer JR, Strom BL, Zauber AG, Stolley PD, Shapiro S. Nonsteroidal anti-inflammatory drugs and risk of digestive cancers at sites other than the large bowel. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 119-123

167 **Wang WH**, Huang JQ, Zheng GF, Lam SK, Karlberg J, Wong BC. Non-steroidal anti-inflammatory drug use and the risk of gastric cancer: a systematic review and meta-analysis. *J Natl Cancer Inst* 2003; **95**: 1784-1791

168 **Kakizoe T**. Cancer Statistics in Japan. Tokyo: Foundation for Promotion of Cancer Research, 1999

169 **Guo HQ**, Guan P, Shi HL, Zhang X, Zhou BS, Yuan Y. Prospective cohort study of comprehensive prevention to gastric cancer. *World J Gastroenterol* 2003; **9**: 432-436

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REVIEW

Stem cells and cancer: Evidence for bone marrow stem cells in epithelial cancers

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Abstract

Cancer commonly arises at the sites of chronic inflammation and infection. Although this association has long been recognized, the reason has remained unclear. Within the gastrointestinal tract, there are many examples of inflammatory conditions associated with cancer, and these include reflux disease and Barrett's adenocarcinoma of the esophagus, Helicobacter infection and gastric cancer, inflammatory bowel disease and colorectal cancer and viral hepatitis leading to hepatocellular carcinoma. There are several mechanisms by which chronic inflammation has been postulated to lead to cancer which includes enhanced proliferation in an endless attempt to heal damage, the presence of a persistent inflammatory environment creating a pro-carcinogenic environment and more recently a role for engraftment of circulating marrow-derived stem cells which may contribute to the stromal components of the tumor as well as the tumor mass itself. Here we review the recent advances in our understanding of the contributions of circulating bone marrow-derived stem cells to the formation of tumors in animal models as well as in human beings.

Key words: Epithelial cancer; Stem cells

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INTRODUCTION

The association between cancer and inflammation has been recognized for over 2 000 years[1,2]. Virchow recognized that many tumors arise in a setting of chronic inflammation, and later, Dvorak aptly described cancer as "wounds that do not heal"[3]. It is estimated that up to 15% of cancers worldwide are associated with chronic infection[4] and there are many more examples throughout the body of cancer associated with inflammation of unclear etiology. Within the GI tract, examples include esophageal adenocarcinoma arising in the setting of Barrett's metaplasia, gastric cancer occurring secondary to Helicobacter infection, colorectal cancer occurring in longstanding inflammatory bowel disease and hepatocellular carcinoma secondary to viral hepatitis (Table 1). There are several mechanisms by which inflammation and a cycle of chronic injury/repair has been postulated to lead to cancer, including the push for continued proliferation, an abnormal inflamed-stromal environment, and more recently we recognize a role for engraftment of circulating marrow-derived stem cells, which may contribute to the stromal components of the tumor as well as the epithelial component of the tumor mass itself.

INFLAMMATION: THE RELATIONSHIP BETWEEN TISSUE INJURY, REPAIR AND CANCER

Proliferation of cells alone is not sufficient to cause cancer, rather proliferation in the setting of altered growth signals from inflammatory cell infiltrates and DNA damaging agents released from infiltrating leukocytes promote malignant degeneration. Wound healing, which requires subversion of usual growth programs, mobilization and migration of cells and a heightened resistance to apoptosis, embodies all the properties of malignant cells with one exception, that is, healing is self limited. Successful wound healing results in restoration of tissue integrity and resolution of inflammation, reinstating homeostatic growth control. The tissue environments of longstanding unrelenting chronic infection or idiopathic chronic inflammation are persistent states of inadequate wound healing. Within this setting, inflammatory cells produce highly reactive oxygen and nitrogen species, which interact with cellular DNA inducing point mutations, deletions or rearrangements. Usually DNA damage such as this triggers

Table 1 Infection and inflammation associated with cancers

Cause	Site
Chewing tobacco/oral irritation	Oral cancers
Smoking/chronic bronchitis	Lung
Asbestos	Mesothelioma
Reflux disease	Barretts' adenocarcinoma of the esophagus
Chronic Helicobacter infection	Gastric adenocarcinoma and lymphoma
Chronic pancreatitis	Pancreatic cancer
Opisthorchis sinensis infection (liver fluke)	Cholangiocarcinoma
Viral hepatitis	Hepatocellular carcinoma
Ulcerative colitis and Crohn's disease	Colorectal carcinoma
Human papilloma virus	Anogenital carcinomas
Schistosomiasis	Bladder cancer
Pelvic inflammatory disease	Ovarian cancer
Chronic osteomyelitis	Osteosarcoma
Chronic scar tissue	"Scar" cancer arising in the lung, skin and other areas of scarring

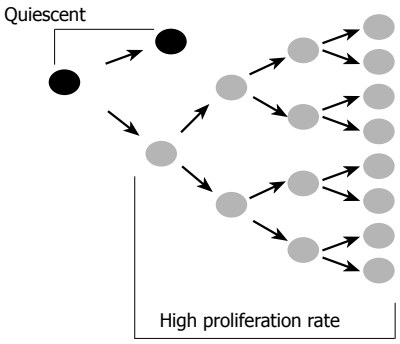

Figure 1 A proposed model for the cancer stem cell. The cancer stem cell replicates itself asymmetrically, thus maintaining one daughter stem cell identical to itself. This remains in a relatively quiescent state. The asymmetric division also produces another daughter cell with a high proliferative rate which rapidly divides to sustain the tumor mass.

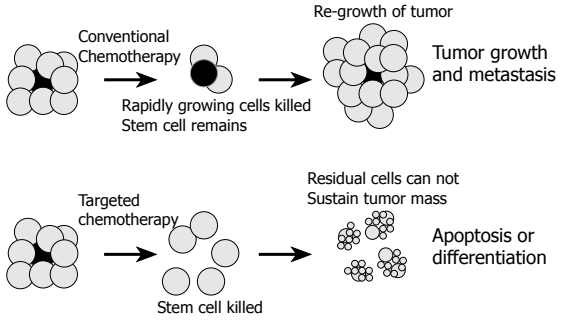

Figure 2 Conventional versus stem cell-targeted chemotherapy. Conventional chemotherapy and radiotherapy targets rapidly dividing cells, and may shrink tumor mass substantially. However, the stem cell (gray), which is relatively quiescent, is not affected. Regrowth of tumor from surviving stem cell leads to regrowth of tumor and treatment failure. Chemotherapy targeted at the stem cell would remove the source of new cell growth, and allow residual cells within the tumor to be targeted with chemotherapy, differentiating agents or therapy aimed at inducing apoptosis, thus successfully eliminating the tumor.

apoptosis. However, in areas of chronic inflammation and repair, growth programs are corrupted and the environment is poised to allow replication and survival of cells, which under normal situations would either be quiescent or lost to apoptosis. Allowing cells to continually divide in an environment conducive to DNA damage may result in the accumulation of genetic defects and the emergence of malignant cells.

Which cell is the target of malignant transformation has been the area of much debate. Original thought was that a terminally differentiated cell would acquire enough genetic damage to replicate endlessly. This would require multiple genetic alterations in key cell signaling cascades to allow autonomous growth. A more likely scenario, however, is that these cells would undergo apoptosis or be sloughed off as a normal part of organ turnover prior to "backing up" the differentiation ladder sufficiently to acquire independent growth. More recently, focus has been on tissue-derived stem cells as the source of cancer. Tissue stem cells possess several important features making them attractive candidates for malignant degeneration. These include long life span, relative apoptosis resistance and ability to replicate for extended periods of time. In the setting of chronic inflammation, progenitor or stem cells within the peripheral tissue are forced to undergo multiple rounds of cell division predisposing to the accumulation of mutations. While restricted progenitors or even differentiated cells can still become transformed, in most cases, it has been believed that early stem cells are the targets of transformation.

If one looks at areas of high proliferative capacity (high BrdU or PCNA staining in tissue) as the stem cell zone, then this theory of peripheral stem cells as the cells of origin of cancer must be reconciled with the fact that this supposed stem cell compartment is often the most damaged and depleted by agents thought to be carcinogens[5]. With chronic inflammation leading to atrophic changes attributed to peripheral stem cell exhaustion, the very cell thought to be transformed is lost - leading us to investigate if an alternate stem cell compartment is responsible for peripheral cancers arising in the setting of inflammation. In order to understand this concept, and why the bone marrow-derived stem cell (BMDC) is a "logical choice" for a cancer stem cell, we need to understand a few points about cancer stem cells, and BMDCs in general.

Stem cells in cancer - cancer as an abnormal organ

A tumor mass can be compared to an abnormal organ; in that it is composed of a heterogeneous mixture of cell types and of cells possessing different proliferative capacities and different levels of differentiation. Tumor cells are admixed with fibroblasts, endothelial cells and inflammatory cells comprising the tumor stroma. This tumor stroma is being increasingly recognized as a critical contributor to malignant growth and survival of the tumor mass; however, stromal cells themselves are usually not malignant. Likewise, not all cells within a tumor are malignant - meaning not all cells can form tumors if transplanted at another site, or into a secondary host. In fact, the majority of cells within a tumor are incapable of independent growth and are readily susceptible to apoptosis. Only a small fraction of cells within a tumor are capable of independent growth, and fulfill the criteria described for cancer stem cells[6-8]. These cells have metastatic potential, form tumors in secondary hosts and are believed to be responsible for continual renewal of cells within the tumor mass. These cells are likely to proliferate slowly and asymmetrically,

self renewing the stem cell population and giving rise to daughter cells, which proliferate to sustain tumor growth (Figure 1). Conventional anti-cancer chemotherapy and radiotherapy target rapidly dividing daughter cells, affecting the bulk of the tumor mass, but leave the cancer stem cell intact, explaining the often rapid recurrence of tumor bulk, once therapy is stopped (Figure 2). At present, the most pressing issue for cancer research is to identify the cancer stem cell and exploit its unique characteristics with targeted therapies.

Bone marrow stem cells

BMDCs are a heterogenous group of cells isolated from the bone marrow which are capable of repopulating the hematopoietic system of a lethally irradiated immunologically compatible secondary host. These cells have been divided into at least two main categories; the hematopoietic and mesenchymal stem cells (MSC). Hematopoietic stem cells are traditionally regarded as the cells which give rise to the formed elements of the blood and have been used extensively in human bone marrow transplantation. Thus, hematopoietic stem cells have been extensively studied and defined with regard to surface markers, growth characteristics and repopulation potential[9]. Less well defined are the MSC. This term MSC, as defined in the literature, is the heterogenous population of cells isolated as the adherent population, when total marrow is placed in culture[10]. These cells give rise to adipocyte, chondrocyte, cells of osteocyte lineages and the marrow mesenchyma, which is vital for optimal hematopoiesis[11-13].

Work from multiple laboratories demonstrates surprising roles for marrow-derived stem cells in addition to hematopoiesis, stressing that the potential for differentiation may be much greater than originally believed. Markers defining cell subpopulations within the marrow are not standardized in these studies, making direct comparison of data between laboratories challenging; however, one thing remains consistent - cells within the bone marrow have a markedly greater differentiation potential than originally believed. For the purposes of this discussion, the term BMDCs will be broadly used to refer to cells derived from the marrow, and will encompass hematopoietic stem cells, MSC multipotent progenitor cells and whole marrow.

In vitro and in vivo studies-plasticity of BMDCs

Multiple and elegant studies from independent groups have shown quite clearly that bone marrow stem cells can differentiate along multiple diverse lineage pathways[14-17]. These findings challenge the conventional view that bone marrow stem cells give rise only to the marrow mesenchyma or formed elements in the peripheral blood. *In vitro*, BMDCs have been shown to differentiate at the single cell level and acquire characteristics of mesoderm, neuroectoderm and endoderm[15,16]. These cells appear to use culture environmental factors to guide lineage decisions. Strikingly, *in vivo* studies in the mouse model have confirmed this plasticity. Elegant studies utilizing transplanted single cells demonstrate differentiation along multiple lineages, supporting a central role for the local tissue environment in dictating differentiation of stem cells, confirming that a single cell is multipotent, and supporting the assertion that experimental findings demonstrating multiple cell lineage differentiation is not due to circulating tissue specific progenitor cells, but rather to a single multipotent cell. In these studies, multiple types of epithelial cells have been shown to be derived from BMDCs including epithelium of the lung, gastrointestinal tract and skin after transplantation of a single bone marrow-derived stem cell[14]. This is not a transient event, as cells can be recovered nearly a year after transplantation. In the gastrointestinal tract, engrafted cells are seen as isolated epithelial cells in the gastric pits of the stomach, the small intestinal villi, the colonic crypt, and rarely in the esophagus. Under these experimental conditions, cells were recovered as single differentiated epithelial cells, and did not appear to engraft into the stem cell niche as clonal expansion was not seen. Infusion of labeled BMDCs into a non-irradiated host, also led to the engraftment (*albeit* to a lesser degree) and differentiation as epithelial cells of the liver, lung and gut in a similar pattern to that seen with marrow ablation and transplantation[15], demonstrating that engraftment and differentiation are true physiological events and not merely artifacts of irradiation and experimental manipulation. While epithelial cell damage is not necessary for engraftment, studies support the notion that damage to the epithelium increases engraftment.

The mechanism by which the marrow-derived cells acquire the appropriate phenotype of epithelial cells is not known, with evidence supporting both direct differentiation or fusion with a peripheral cell[18-21]. The method of engraftment and differentiation may be specific to the individual tissues and/or may depend on the mechanism of injury inducing engraftment. Irrespective of the mechanism involved, BMDCs have been shown to engraft and take on the function of cells within the peripheral tissues[16,18-23].

HUMAN STUDIES - EVIDENCE FOR PLASTICITY OF BMDCS IN PATIENTS TRANSPLANTED WITH GENDER MISMATCHED BONE MARROW

Human studies have confirmed that plasticity of BMDCs is not restricted to mice, and may be a physiologically relevant phenomenon in man as well. Studies, examining peripheral tissue of female patients transplanted with bone marrow derived from male donors, have shown that BMDCs from the donor can differentiate into skin, gut epithelium and mature hepatocytes[24,25]. Identification of the Y chromosome in cells of these tissues confirms that BMDCs can substantially repopulate the GI tract epithelium[25], and this repopulation does not appear to be a rare event. Patients in these studies have some level of graft-versus-host disease, and the level of inflammation in the tissue correlates with the level of donor cell engraftment. These findings are consistent with the data derived from murine studies and suggest the inflammatory environment is crucial for optimal engraftment and differentiation of BMDCs. The fact that BMDCs have the capacity to differentiate along organ-specific lineages appropriate for the organ of engraftment, and are found in increasing num-

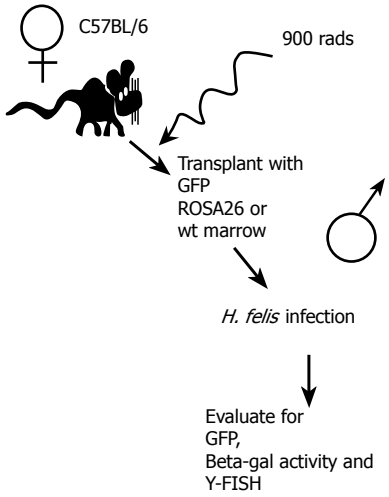

Figure 3 An experimental mouse model for bone marrow transplantation and *H. felis*-induced gastric carcinoma.

bers during chronic inflammation (a condition associated with cancer), places these cells "in the right place, at the right time" to be candidates for the cancer stem cell.

Similarities between BMDCs and cancer cells

In addition to what appears to be of immense plasticity of cells within the bone marrow, BMDCs have other traits which make them attractive candidates for cancer stem cells. BMDCs have the capacity for self renewal, are long lived, are chemoresistant, and may be inherently mutagenic[26-29]. Intriguing is the fact that similar growth regulators and control mechanisms are involved in both cancer and stem cell maintenance. For example, proteins from the polycomb group, the epigenetic chromatin modifiers, are involved in both cancer development and maintenance of embryonic and adult stem cells[30]. Also, pathways used by bone marrow stem cells for trafficking appear to be exploited by tumor cells for metastasis[31]. For instance, chemokines and cytokines produced during chronic inflammation (such as SDF-1) influence the behavior and migration of cancer cells. These are the same chemokines and cytokines responsible for physiological stem cells homing back to the marrow cavity[32-36]. Identification of bone marrow stromal cell-derived growth inhibitor as a potent inhibitor of breast cancer cell migration, and the capability of this protein to induce cell cycle arrest and apoptosis in breast cancer stem cells further supports the use of similar growth mechanisms between stem and cancer cells[37]. Inflammation of the GI tract is associated with IL-6 and IL-8 production which initiate neutrophil infiltration[39]. Interestingly, IL-6 is also chemotactic for MSC[33]. Other cytokines and chemokines prominent in the setting of mucosal inflammation such as VEGF and MIP-1α are also chemoattractants for MSC[33,34]. Receptors such as CXCR 2 and 4 are found on both cancer cells and stem cells, and influence the homing of stem cells, or invasion/metastases of cancer cells, suggesting a link between the two populations of cells. One might suppose that a mechanism similar to that used to regulate BMDC circulation and homing back to areas of bone may also facilitate migration and engraftment of BMDCs into peripheral tissues as a result of chronic inflammation, if the peripheral tissue secretes the appropriate homing signals.

Additionally, immune escape has long been a perplexing property of cancer cells; MSCs have unique immunological properties in that they are not immunogenic, they do not stimulate alloreactivity, and they escape lysis by cytotoxic T cells and natural killer cells[39]. This inherent ability to evade immune recognition may explain why many cancer cells evade the host immune response.

BMDCs as the origin of epithelial cancer: helicobacter induced gastric cancer as a model system

We reasoned that BMDCs, as the ultimate uncommitted adult stem cell, might represent the ideal candidate for transformation, if placed in a favorable environment. We used the well-described *H. felis*/C57BL/6 mouse model of gastric cancer to test this theory[40]. This model is optimal for studying the role of stem cells in inflammatory-mediated cancers because C57BL/6 mice do not develop gastric cancer under controlled conditions. With Helicobacter infection, however, the gastric mucosa progresses through a series of changes including metaplasia and dysplasia, culminating in gastrointestinal intraepithelial neoplasia (GIN)[41] by 12-15 mo of infection, thus reiterating human disease, where gastric cancer in the absence of Helicobacter infection is unusual, while longstanding infection carries a significant (up to 1-3%) risk of gastric cancer[42-47]. In order to test the role for BMDCs in gastric cancer (Figure 3), C57BL/6J mice were myeloablated and transplanted with gender-mismatched bone marrow from mice that expressed a non-mammalian beta-galactosidase enzyme [C57BL/*6JGtrosa26* (ROSA 26)], mice that expressed green fluorescent protein [C57BL/6J-*beta-actin-EGFP* (GFP)], or control C57BL/6J liter mates. Engraftment of ROSA26 BMDCs into the gastric mucosa was confirmed by several independent methods including detecting enzyme activity, specific B-galactosidase immunohistochemistry (IHC, two cytoplasmic markers) (Figures 4 and 5), and detection of LacZNeo fusion gene sequence (nuclear marker) by PCR within beta-galactosidase positive gastric glands isolated by laser capture microscopy. In those mice transplanted with GFP marrow, GFP was detected by fluorescence activated cell sorting of cytokeratin positive-single cell preparations, and GFP immunohistochemistry of tissue sections. X and Y chromosome fluorescent *in situ* hybridization (X and Y-FISH) was used as an additional means to detect BMDCs in gender mismatched transplants[40].

As expected, acute Helicobacter infection was associated with an influx of bone marrow-derived inflammatory cells (Figure 4A - blue staining) into the tissue. At early time points, we did not detect any engraftment or differentiation of BMDCs to an epithelial cell phenotype. At 20 wk of infection, rare glands entirely replaced by BMDCs were isolated, suggesting that engraftment into the stem cell niche had occurred. These findings were more pronounced at 30 wk, where antralized glands and metaplastic cells at the squamocolumnar junction were entirely replaced by marrow-derived cells (Figures 4D and 4E). The severity of intraepithelial dysplasia increased over time, and by one year of infection, most mice developed invasive neoplastic glands. All of the intraepithelial neoplasia in mice infected

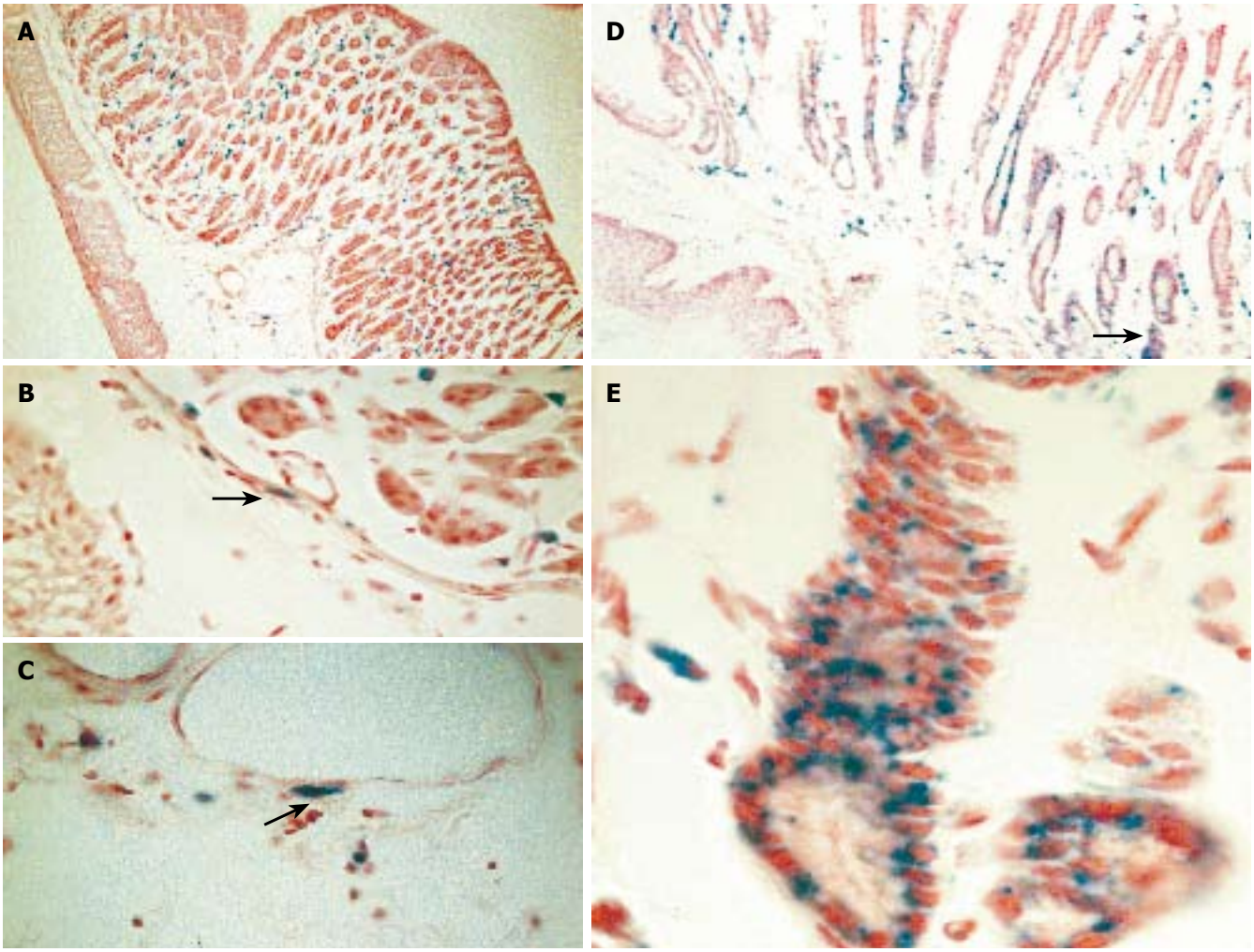

Figure 4 Engraftment of donor-derived ROSA-26 marrow by x-gal staining. **A:** Mice transplanted with ROSA 26 marrow and infected with *H. felis* for 4 wk had donor-derived leukocytes (blue) infiltrating the gastric mucosa, and no engraftment into gland structures. **B** and **C:** A higher power view reveals myocytes and myofibroblasts in the submucosal tissue adjacent to vascular structures (arrows). **D:** After 30 wk of infection, marked architectural distortion is seen with antralization and appearance of metaplastic glands. Entire gland structures are derived from donor marrow (blue staining). Gland shown in panel D (arrow) is shown at higher power in **E**.

for 12-16 mo rose from donor marrow cells, strongly suggesting an inherent vulnerability of this population of cells to malignant progression. Progressive parietal and chief cell loss is a hallmark of chronic Helicobacter infection. Of the few parietal or chief cells which we isolated from the infected mice, none were derived from the bone marrow, strongly suggesting that marrow cells do not differentiate toward the parietal or chief cell phenotype under the experimental conditions that were used[40].

Normal healing of the gastric mucosa after iatrogenic ulceration likewise did not require BMDCs[40], nor did loss of specific cell lineages, such as targeted ablation of parietal cells, lead to marrow engraftment[40]. Rather, it seems that long standing inflammation and inflammatory mediated damage to the epithelium is required - an environment strongly linked to the development of cancer in many settings. In our Helicobacter-gastric cancer model, infection and inflammation reached a plateau at 8 wk; however, engraftment was not apparent until 20 wk, suggesting that events other than increased inflammation are responsible for engraftment. Between 8 and 20 wk, there is loss of the oxyntic glands, and a restructuring of the gastric architecture to include metaplastic cell lineages, reflecting the effects of an abnormal tissue milieu on rapidly proliferating cells[48]. Once engraftment began, however, the number of bone marrow-derived glands increased dramatically, suggesting that a threshold for recruitment had been reached[40].

In addition to epithelial cells within the tumor, BMDCs also comprise a subset of cells within the tumor stroma and within seemingly uninvolved epithelium and subepithelial spaces adjacent to the tumors. We have recovered adipocytes (Figure 5C), fibroblast, endothelial cells and myofibroblasts (Figures 4B and 4C) derived from bone marrow precursors in areas adjacent to dysplasia and neoplasia.

Based on these experiments, we have proposed a new paradigm for epithelial cancer (Figure 6). Chronic tissue inflammation leads to tissue injury and with time, to tissue stem cell failure. Peripheral stem cell failure leads to recruitment and permanent engraftment of BMDCs into the tissue stem cell niche, where the BMDCs essentially take over the function of the tissue stem cell. In the setting of inflammation, specifically with Th1 type cytokines and an abnormal tissue environment (for example, one lacking chief and parietal cells), the BMDCs initiate differentia-

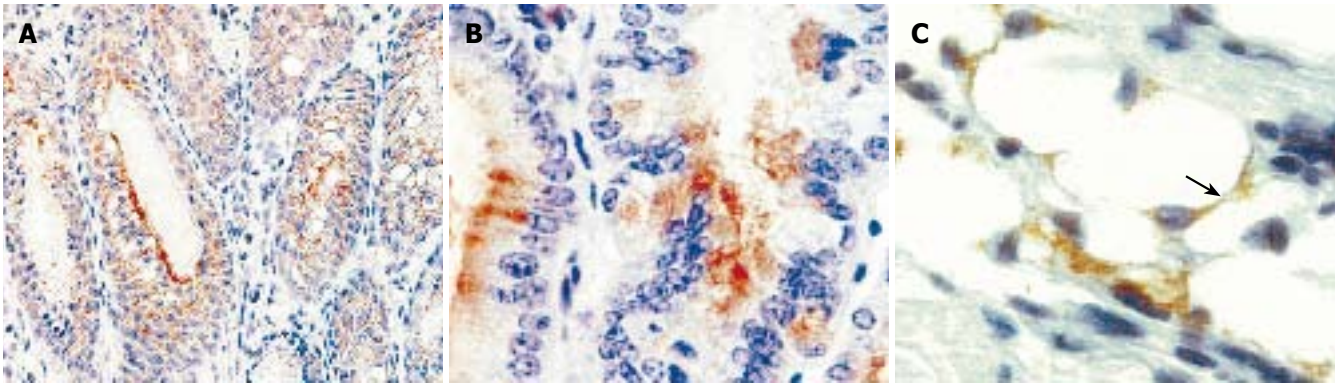

Figure 5 Immunohistochemistry for bacterial beta-galactosidase confirms uniform signal in gastrointestinal neoplasia. Mice developed severe dysplasia and intraepithelial neoplasia derived from donor marrow, 12-15 mo after infection with *H. felis* (**A**) and (**B**). Immunohistochemistry for bacterial beta-galactosidase demonstrates cytoplasmic staining in dysplastic glands. A population of adipocytes in the submucosa are also stained for beta-galactosidase (arrow).

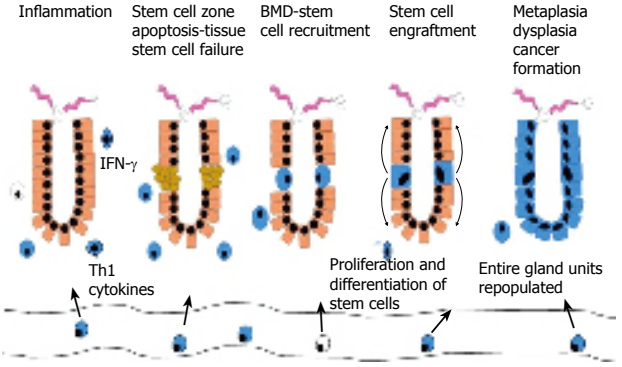

Figure 6 A new paradigm proposed for epithelial cancer.

tion, but fail to regulate growth programs appropriately and progresses through stages of metaplasia and dysplasia. We speculate that the inappropriate retention of primitive growth programs in a stem cell forced to replicate may permit survival despite otherwise lethal mutations, thus allowing transformation. This new model brings together previously unexplained observations regarding the behavior of cancer, and presupposes that properties inherent to cancer such as their resistance to apoptosis, their unlimited growth potential and their ability for local spread and distant metastasis are fundamental to the origin of the cell, rather than traits acquired. The concept of cancer initiation and promotion can also be viewed within the context of this model. Initiation may represent BMDCs trafficking into the stem cell niche as a result of tissue stem cell damage. In the absence of continued inflammation and injury, these engrafted cells may behave in a way indistinguishable from endogenous tissue stem cells. Promotion may represent an additional stimulus received at a later time that allows sustained proliferation of BMDCs and transformation.

In vitro experiments and animal models supporting the BMDC-epithelial cancer model

In addition to the Helicobacter-gastric cancer model, other studies have begun to address the role of BMDCs in cancer using various *in vitro* and *in vivo* models. For example, BMDCs have been shown to localize to a known stem cell niche within the epidermis known as the CD34 positive bulge region of the hair follicle, and clonally expand to repopulate portions of the epidermis, functioning as an epidermal stem cell[49]. Similar to our findings, engraftment of BMDCs to the stem cell niche is dramatically increased with injury severe enough to deplete peripheral stem cells in the region. However, these are short-term studies. Longer term studies utilizing carcinogen exposure will determine the eventual fate of these BMDCs, and determine if BMDCs in the stem cell niche behave differently from peripheral stem cells occupying the same niche. It is intriguing, however, to speculate the ultimate fate of these stem cells given the prevalence of BMDC-skin carcinoma in solid organ recipients (see below).

In addition to residing in the epithelial stem cell niche, bone marrow-derived myofibroblasts have been recovered within the colonic subepithelial compartments in both mice and human beings[50,51]. Interestingly, Direkze *et al.* observed that in the IL-10 knockout mouse model of colitis, up to 45% of subepithelial myofibroblasts were marrow derived[51], suggesting that in the setting of chronic inflammation, damaged tissue is replaced by BMDCs. When the same group looked at tumor-associated myofibroblasts and fibroblasts, they also found a significant portion of these cells derived from bone marrow cells[50]. It is not clear from these data, if tumors recruit bone marrow cells into the stromal compartment or if resident myofibroblasts and fibroblasts derived from marrow contribute to tumor formation because of abnormal signaling behavior.

Adenocarcinoma of the distal esophagus (Barretts' adenocarcinoma) results from reflux-induced mucosal damage followed by healing with a metaplastic intestinal cell lineage. This intestinal metaplasia is prone to malignant degeneration and is another ideal model to test the role of BMDCs in inflammatory-mediated cancers. Using a rat model of Barrett's metaplasia, a significant contribution of BMDCs to the stroma and the metaplastic epithelium has been demonstrated, supporting a role for BMDCs in these pre-neoplastic lesions[52]. Though these findings have only been reported in an abstract form so far, this information is especially exciting because it provides evidence of direct BMDC involvement in carcinogenesis from both an additional species (rat) and tissue type (esophagus), providing

further support for our BMDC-epithelial cancer model.

Human data supporting the BMDC-epithelial cancer model

In human beings, the incidence of solid tumors is significantly increased following bone marrow transplantation[53] and may be related to persistent chronic inflammation of graft *vs.* host disease. The data on BMDCs in human cancers, however, have been conflicting. First, it is difficult to examine the contribution of donor marrow to tumor formation in human beings because of a paucity of cell markers to consistently identify autologous BMDCs or donor cells after BM transplantation. The most reliable marker we have to date is identification of the sex chromosomes in sex mismatched transplants. However, there are inherent difficulties with using Y-chromosome identification. X/Y fluorescent *in situ* hybridization (FISH) analysis of archived tumors is estimated to miss more than 50% of Y-positive cells due to sectioning bias, where only a portion of the nucleus and thus only a portion of the chromosomes are included in the tissue section. Additionally, females with a history of carrying a male fetus may show peripheral blood chimerism confounding interpretation of data, and eliminating this population from the study. Also, tumors identified within a short time after transplant may reflect the effects of immunosuppression on previously undetected early malignancy and not newly formed tumors, and may explain why some studies conclude tumors in these patients are host derived[54], while other studies demonstrate a definite contribution of donor's-BMDCs[55]. Studies utilizing larger numbers of patients followed for longer periods of time will better address this new and controversial area, and determine if the BMDCs are confined to the stroma, involved in angiogenesis or constitute the epithelial component of the tumor mass in human beings.

In addition to patients receiving bone marrow transplants, recipients of solid organ transplants also have a higher incidence of secondary malignancy. Interestingly, in solid organ transplant recipients, hematopoietic cells of donor origin are often found in the circulation, indicating that hematopoietic stem cells are transferred with the transplanted organ[56,57]. These transferred stem cells have been shown to give rise to Kaposi sarcoma (KS), a vascular tumor[58], and skin carcinoma[59]. The detected KS lesions occurred distal to the graft site, and formed presumably via mobilization of donor progenitor cells with subsequent transformation at a distant site. Donor-derived stem cells contribute to skin carcinomas, and have been recovered as components of squamous cell carcinoma, basal cell carcinoma, actinic keratosis, keratoacanthomas and benign cutaneous lesions[59], attesting to the great potential for abnormal differentiation of these cells. BMDCs as terminally differentiated cells in other organs including hepatic endothelial cells, hepatocytes and biliary epithelial cells[60], suggesting that these cells may play a role in transformation within these organs as well, if subjected to the appropriate environmental conditions.

CONCLUSION

One of the greatest and most elusive challenges in cancer biology has been to identify the cellular origin of cancer. We have identified the bone marrow stem cell as the cell of origin of Helicobacter-induced gastric cancer in a mouse model, radically altering our current view of gastric cancer formation in particular, and of inflammation-mediated cancers in general. The concept of BMDC plasticity is being increasingly recognized and validated by independent groups. Our recent observation that BMDCs are the origin of Helicobacter-induced gastric cancer[40] combined with supporting observations of BMDCs in other tumors such as benign and malignant tumors of the skin[59], Kaposis sarcoma[58] Barretts' adenocarcinoma of the esophagus[52] as well as demonstration of BMDCs as constituents of tumor stroma and tumor vascular structures[51-55] suggests exciting approaches for cancer therapy. If the propensity for BMDCs to transform is based on inappropriate regulation of immature growth programs, with growth programs left "turned on" rather than the previously held concept of mutation driven-reactivation of programs, can we target these pathways? Undoubtedly, genetic mutations have occurred which are irreversible; but if we can target and switch off inappropriately activated growth cascades, perhaps we can push these damaged cells into apoptosis or enhance the sensitivity to conventional chemo- and radiotherapy. These approaches may lead to novel and more efficacious cancer therapy.

Presently, our laboratory is involved in identifying the cell population within the bone marrow capable of cancer formation as well as defining the homing and differentiation signals which allow these cells to access to gastric mucosa, and to differentiate as metaplastic and dysplastic cells. Studies designed to determine if fusion is a means of bone marrow cell integration into gastric mucosa and gastric cancer are underway. The applicability of these findings to other epithelial cancers will be tested as well as our ability to control the growth of these cells by manipulations of the local tissue environment. These efforts are aimed at identifying cell-specific targets for chemotherapy. Findings from these studies will radically alter our approach to the treatment of gastric cancer as well as other solid tumors, and offer hope for improved survival and potential cure.

REFERENCES

1 **Balkwill F**, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539-545
2 **Coussens LM**, Werb Z. Inflammation and cancer. *Nature* 2002; **420**: 860-867
3 **Dvorak HF**. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med* 1986; **315**: 1650-1659
4 **Kuper H**, Adami HO, Trichopoulos D. Infections as a major preventable cause of human cancer. *J Intern Med* 2000; **248**: 171-183
5 **Potten CS**, Booth C, Hargreaves D. The small intestine as a model for evaluating adult tissue stem cell drug targets. *Cell Prolif* 2003; **36**: 115-129
6 **Bruce WR**, Van der gaag H. A quantitative assay for the number of murine lymphoma cells capable of proliferation in vivo. *Nature* 1963; **199**: 79-80
7 **Hamburger AW**, Salmon SE. Primary bioassay of human tumor stem cells. *Science* 1977; **197**: 461-463
8 **Park CH**, Bergsagel DE, McCulloch EA. Mouse myeloma tumor stem cells: a primary cell culture assay. *J Natl Cancer*

Inst 1971; **46**: 411-422
9 **Baum CM**, Weissman IL, Tsukamoto AS, Buckle AM, Peault B. Isolation of a candidate human hematopoietic stem-cell population. *Proc Natl Acad Sci U S A* 1992; **89**: 2804-2808
10 **Baddoo M**, Hill K, Wilkinson R, Gaupp D, Hughes C, Kopen GC, Phinney DG. Characterization of mesenchymal stem cells isolated from murine bone marrow by negative selection. *J Cell Biochem* 2003; **89**: 1235-1249
11 **Bensidhoum M**, Chapel A, Francois S, Demarquay C, Mazurier C, Fouillard L, Bouchet S, Bertho JM, Gourmelon P, Aigueperse J, Charbord P, Gorin NC, Thierry D, Lopez M. Homing of in vitro expanded Stro-1- or Stro-1+ human mesenchymal stem cells into the NOD/SCID mouse and their role in supporting human CD34 cell engraftment. *Blood* 2004; **103**: 3313-3319
12 **Kassem M**. Mesenchymal stem cells: biological characteristics and potential clinical applications. *Cloning Stem Cells* 2004; **6**: 369-374
13 **Bai X**, Xiao Z, Pan Y, Hu J, Pohl J, Wen J, Li L. Cartilage-derived morphogenetic protein-1 promotes the differentiation of mesenchymal stem cells into chondrocytes. *Biochem Biophys Res Commun* 2004; **325**: 453-460
14 **Krause DS**, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. *Cell* 2001; **105**: 369-377
15 **Jiang Y**, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 2002; **418**: 41-49
16 **LaBarge MA**, Blau HM. Biological progression from adult bone marrow to mononucleate muscle stem cell to multinucleate muscle fiber in response to injury. *Cell* 2002; **111**: 589-601
17 **Petersen BE**, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP. Bone marrow as a potential source of hepatic oval cells. *Science* 1999; **284**: 1168-1170
18 **Newsome PN**, Johannessen I, Boyle S, Dalakas E, McAulay KA, Samuel K, Rae F, Forrester L, Turner ML, Hayes PC, Harrison DJ, Bickmore WA, Plevris JN. Human cord blood-derived cells can differentiate into hepatocytes in the mouse liver with no evidence of cellular fusion. *Gastroenterology* 2003; **124**: 1891-1900
19 **Harris RG**, Herzog EL, Bruscia EM, Grove JE, Van Arnam JS, Krause DS. Lack of a fusion requirement for development of bone marrow-derived epithelia. *Science* 2004; **305**: 90-93
20 **Vassilopoulos G**, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. *Nature* 2003; **422**: 901-904
21 **Wang X**, Willenbring H, Akkari Y, Torimaru Y, Foster M, Al-Dhalimy M, Lagasse E, Finegold M, Olson S, Grompe M. Cell fusion is the principal source of bone-marrow-derived hepatocytes. *Nature* 2003; **422**: 897-901
22 **Camargo FD**, Green R, Capetanaki Y, Jackson KA, Goodell MA. Single hematopoietic stem cells generate skeletal muscle through myeloid intermediates. *Nat Med* 2003; **9**: 1520-1527
23 **Shimizu K**, Sugiyama S, Aikawa M, Fukumoto Y, Rabkin E, Libby P, Mitchell RN. Host bone-marrow cells are a source of donor intimal smooth- muscle-like cells in murine aortic transplant arteriopathy. *Nat Med* 2001; **7**: 738-741
24 **Yeh ET**, Zhang S, Wu HD, Körbling M, Willerson JT, Estrov Z. Transdifferentiation of human peripheral blood CD34+-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo. *Circulation* 2003; **108**: 2070-2073
25 **Körbling M**, Katz RL, Khanna A, Ruifrok AC, Rondon G, Albitar M, Champlin RE, Estrov Z. Hepatocytes and epithelial cells of donor origin in recipients of peripheral-blood stem cells. *N Engl J Med* 2002; **346**: 738-746
26 **Serakinci N**, Guldberg P, Burns JS, Abdallah B, Schrødder H, Jensen T, Kassem M. Adult human mesenchymal stem cell as a target for neoplastic transformation. *Oncogene* 2004; **23**: 5095-5098
27 **Rubio D**, Garcia-Castro J, Martín MC, de la Fuente R, Cigudosa JC, Lloyd AC, Bernad A. Spontaneous human adult stem cell transformation. *Cancer Res* 2005; **65**: 3035-3039
28 **Xu W**, Qian H, Zhu W, Chen Y, Shao Q, Sun X, Hu J, Han C, Zhang X. A novel tumor cell line cloned from mutated human embryonic bone marrow mesenchymal stem cells. *Oncol Rep* 2004; **12**: 501-508
29 **Burns JS**, Abdallah BM, Guldberg P, Rygaard J, Schrøder HD, Kassem M. Tumorigenic heterogeneity in cancer stem cells evolved from long-term cultures of telomerase-immortalized human mesenchymal stem cells. *Cancer Res* 2005; **65**: 3126-3135
30 **Valk-Lingbeek ME**, Bruggeman SW, van Lohuizen M. Stem cells and cancer; the polycomb connection. *Cell* 2004; **118**: 409-418
31 **Liang Z**, Wu T, Lou H, Yu X, Taichman RS, Lau SK, Nie S, Umbreit J, Shim H. Inhibition of breast cancer metastasis by selective synthetic polypeptide against CXCR4. *Cancer Res* 2004; **64**: 4302-4308
32 **Lyden D**, Hattori K, Dias S, Costa C, Blaikie P, Butros L, Chadburn A, Heissig B, Marks W, Witte L, Wu Y, Hicklin D, Zhu Z, Hackett NR, Crystal RG, Moore MA, Hajjar KA, Manova K, Benezra R, Rafii S. Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. *Nat Med* 2001; **7**: 1194-1201
33 **Rodríguez Mdel C**, Bernad A, Aracil M. Interleukin-6 deficiency affects bone marrow stromal precursors, resulting in defective hematopoietic support. *Blood* 2004; **103**: 3349-3354
34 **Toh K**, Kukita T, Wu Z, Kukita A, Sandra F, Tang QY, Nomiyama H, Iijima T. Possible involvement of MIP-1alpha in the recruitment of osteoclast progenitors to the distal tibia in rats with adjuvant-induced arthritis. *Lab Invest* 2004; **84**: 1092-1102
35 **El-Omar EM**, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, Stanford JL, Mayne ST, Goedert J, Blot WJ, Fraumeni JF, Chow WH. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology* 2003; **124**: 1193-1201
36 **Janowska-Wieczorek A**, Marquez LA, Nabholtz JM, Cabuhat ML, Montaño J, Chang H, Rozmus J, Russell JA, Edwards DR, Turner AR. Growth factors and cytokines upregulate gelatinase expression in bone marrow CD34(+) cells and their transmigration through reconstituted basement membrane. *Blood* 1999; **93**: 3379-3390
37 **Wang T**, Xia D, Li N, Wang C, Chen T, Wan T, Chen G, Cao X. Bone marrow stromal cell-derived growth inhibitor inhibits growth and migration of breast cancer cells via induction of cell cycle arrest and apoptosis. *J Biol Chem* 2005; **280**: 4374-4382
38 **Suzuki T**, Ina K, Nishiwaki T, Tsuzuki T, Okada T, Furuta R, Nobata K, Ando T, Kusugami K, Goto H. Differential roles of interleukin-1beta and interleukin-8 in neutrophil transendothelial migration in patients with Helicobacter pylori infection. *Scand J Gastroenterol* 2004; **39**: 313-321
39 **Reya T**, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001; **414**: 105-111
40 **Houghton J**, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Cai X, Fox JG, Goldenring JR, Wang TC. Gastric cancer originating from bone marrow-derived cells. *Science* 2004; **306**: 1568-1571
41 **Boivin GP**, Washington K, Yang K, Ward JM, Pretlow TP, Russell R, Besselsen DG, Godfrey VL, Doetschman T, Dove WF, Pitot HC, Halberg RB, Itzkowitz SH, Groden J, Coffey RJ. Pathology of mouse models of intestinal cancer: consensus report and recommendations. *Gastroenterology* 2003; **124**: 762-777
42 **Nomura A**, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med* 1991; **325**: 1132-1136
43 **Correa P**. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res*

1992; **52**: 6735-6740
44 Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1983; **1**: 1273-1275
45 **Forman D**, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sitas F. Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. *BMJ* 1991; **302**: 1302-1305
46 **Parsonnet J**, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131
47 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789
48 **Cai X**, Carlson J, Stoicov C, Li H, Wang TC, Houghton J. Helicobacter felis eradication restores normal architecture and inhibits gastric cancer progression in C57BL/6 mice. *Gastroenterology* 2005; **128**: 1937-1952
49 **Brittan M**, Braun KM, Reynolds LE, Conti FJ, Reynolds AR, Poulsom R, Alison MR, Wright NA, Hodivala-Dilke KM. Bone marrow cells engraft within the epidermis and proliferate in vivo with no evidence of cell fusion. *J Pathol* 2005; **205**: 1-13
50 **Bamba S**, Otto WR, Lee CY, Brittan M, Preston SL, Wright NA. The contribution of bone marrow to colonic subepithelial myofibroblasts in interleukin -10 knock out mice. *Gastroenterology* 2005; **128**: A490
51 **Direkze NC**, Hodivala-Dilke K, Jeffery R, Hunt T, Poulsom R, Oukrif D, Alison MR, Wright NA. Bone marrow contribution to tumor-associated myofibroblasts and fibroblasts. *Cancer Res* 2004; **64**: 8492-8495
52 **Sarosi GA**, Brown G, Jaiswal K, Lee E, Crook T, Souza R, Zou Y, Shat J, Spechler S. Reflux-Damaged Esophageal Epithelium Is Replaced By Cells Derived from the Bone Marrow in a Rat Model of Barrett's Esophagus. *Gastroenterology* 2004; **126**: A35
53 **Adès L**, Guardiola P, Sociè G. Second malignancies after allogeneic hematopoietic stem cell transplantation: new insight and current problems. *Blood Rev* 2002; **16**: 135-146
54 **Au WY**, Chan EC, Pang A, Lie AK, Liang R, Yuen AP, Shek TW, Kwong YL. Nonhematologic malignancies after allogeneic hematopoietic stem cell transplantation: incidence and molecular monitoring. *Bone Marrow Transplant* 2004; **34**: 981-985
55 **Peters BA**, Diaz LA, Polyak K, Meszler L, Romans K, Guinan EC, Antin JH, Myerson D, Hamilton SR, Vogelstein B, Kinzler KW, Lengauer C. Contribution of bone marrow-derived endothelial cells to human tumor vasculature. *Nat Med* 2005; **11**: 261-262
56 **Suberbielle C**, Caillat-Zucman S, Legendre C, Bodemer C, Noël LH, Kreis H, Bach JF. Peripheral microchimerism in long-term cadaveric-kidney allograft recipients. *Lancet* 1994; **343**: 1468-1469
57 **Sivasai KS**, Alevy YG, Duffy BF, Brennan DC, Singer GG, Shenoy S, Lowell JA, Howard T, Mohanakumar T. Peripheral blood microchimerism in human liver and renal transplant recipients: rejection despite donor-specific chimerism. *Transplantation* 1997; **64**: 427-432
58 **Barozzi P**, Luppi M, Facchetti F, Mecucci C, Alù M, Sarid R, Rasini V, Ravazzini L, Rossi E, Festa S, Crescenzi B, Wolf DG, Schulz TF, Torelli G. Post-transplant Kaposi sarcoma originates from the seeding of donor-derived progenitors. *Nat Med* 2003; **9**: 554-561
59 **Aractingi S**, Kanitakis J, Euvrard S, Le Danff C, Peguillet I, Khosrotehrani K, Lantz O, Carosella ED. Skin carcinoma arising from donor cells in a kidney transplant recipient. *Cancer Res* 2005; **65**: 1755-1760
60 **Hove WR**, van Hoek B, Bajema IM, Ringers J, van Krieken JH, Lagaaij EL. Extensive chimerism in liver transplants: vascular endothelium, bile duct epithelium, and hepatocytes. *Liver Transpl* 2003; **9**: 552-556

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REVIEW

Current role of surgical therapy in gastric cancer

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Abstract

Surgery is currently the only potentially curative treatment for gastric cancer. Since the inception of the gastrectomy for cancer of the stomach, there has been debate over the bounds of surgical therapy, balancing potential long-term survival with perioperative morbidity and mortality. This review delineates the current role of surgery in preoperative staging, curative resection, and palliative treatment for gastric cancer.

Key words: Gastric; Cancer; Surgery

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INTRODUCTION

Gastric cancer is one of the most common malignancies in the world and is a leading cause of cancer death. Despite some recent advances in neoadjuvant therapy, studies generally have failed to show any improvement in overall or relapse-free survival following adjuvant therapy. Surgical therapy remains as the most effective modality in treating gastric cancer. The goal of this review is to evaluate the optimal role and impact of surgery in regards to staging, resection of primary, regional and locally advanced disease, and palliation of symptoms in patients with incurable gastric cancer.

STAGING

Laparoscopy has emerged as an essential staging modality prior to gastric resection, identifying unresectable disease in a significant number of patients deemed resectable by current radiographic and endoscopic modalities. The diagnostic yield of laparoscopy has been improved by the addition of laparoscopic ultrasound and peritoneal cytology.

Stell *et al*[1] compared 103 patients with gastric adenocarcinoma who underwent preoperative staging with ultrasound (US), computed tomography (CT), and laparoscopy. Histologic confirmation was obtained at laparotomy ($n = 65$) or with percutaneous liver biopsy during laparoscopy ($n = 27$). The sensitivity, specificity, and accuracy of laparoscopy in detecting hepatic metastases ($n = 27$) was 96%, 100%, and 99%, respectively. Laparoscopy had a sensitivity of 53%, a specificity of 100%, and an accuracy of 65% in detecting nodal metastasis ($n = 49$). Peritoneal metastases were histologically confirmed in 13 patients, and laparoscopy was 69% sensitive, 100% specific, and 94% accurate.

Burke *et al*[2] reviewed 103 patients who were deemed free from intra-abdominal metastases by CT scan and subsequently underwent laparoscopy. A control group who underwent laparotomy for possible gastric resection and subsequently found to have M1 disease was also reviewed. Laparoscopy accurately staged 94% of these patients. Histologic evidence of metastasis was obtained in 32 patients during laparoscopy. Of the 71 patients who were M0 by laparoscopy, 65 were confirmed by laparotomy. Of the six false negatives, three were missed peritoneal metastases, and three were missed distant nodal metastases, two of which were identified after resection. Of the 32 patients who were M1 on laparoscopy, 4 had chemotherapy followed by resection, 3 had further palliative procedures, and 1 had a mini-laparotomy for additional specimen. The remaining 24 patients went on to have no further operation. When comparing these 24 patients to the control group of 60 patients with M1 disease on laparotomy, the hospital stay was significantly shorter. There was one complication in the laparoscopy group and eight in the laparotomy group; however, this did not reach significance. The authors concluded that laparoscopy is a valuable staging tool that can spare patients with asymptomatic M1 disease a laparotomy, and identify those patients who may benefit from neoadjuvant therapy.

The addition of laparoscopic US has improved the yield of laparoscopy for staging gastric cancer. Hulscher *et al*[3] reviewed the utility of laparoscopic US in staging 48 patients with gastric carcinoma of the cardia and esophageal invasion. All of these patients were staged with endoscopic US, percutaneous US of the neck and abdomen, conventional chest radiograph and bronchoscopy if there was extensive proximal invasion. Metastatic

disease was detected and histologically confirmed in 11 patients (23%), 7 detected with laparoscopy alone, and an additional 4 detected by laparoscopic US. Of the patients who went on to laparotomy, two were found to have posterior liver metastases, and two to have unresectable lymph nodes at the base of the celiac axis. Three of these four lesions were detected laparoscopically, but were either not amenable to biopsy due to position, or were negative on laparoscopic biopsy. The authors concluded that laparoscopy is a valuable staging tool for gastric adenocarcinoma of the cardia, and that the addition of US increases the yield, avoiding laparotomy in 23% of patients.

Peritoneal cytology obtained at laparoscopy has proven to be a predictor of post-operative mortality in patients undergoing curative (R0) resection. Bentrem *et al*[4] reviewed 371 patients who underwent staging laparoscopy with peritoneal washings and subsequent R0 resection as a combined procedure. Twenty-four patients (6%) had positive cytology, defined as the presence of adenocarcinoma cells, regardless of quantity. Positive cytology without evidence of macroscopic M1 disease was associated with advanced preoperative T stage and advanced AJCC stage, but not preoperative N stage. The median survival for patients with positive cytology was 15 mo, compared to 98.5 mo for those with negative cytology. On multivariate analysis, preoperative T stage, preoperative N stage, primary tumor site, and peritoneal cytology were significant predictors of survival, with positive cytology showing the highest risk ratio. The authors have altered their practice in response to this information, performing staging laparoscopy as a separate procedure in patients considered high risk (T3/4 and AJCC stage II/III). Those patients found to have positive cytology were then referred for chemotherapy followed by possible resection with intraperitoneal chemotherapy.

Laparoscopy and laparoscopy US are currently accepted as standard pre-operative staging tools. Laparoscopy is significantly more accurate than US and CT in detecting hepatic, nodal and peritoneal metastases. Reports suggest minimal added morbidity and no mortality from laparoscopy. Peritoneal cytology is not widely accepted as a screening tool; however, this is a topic of growing research interest.

EXTENT OF GASTRIC RESECTION

Distal gastric adenocarcinoma

The extent of gastric resection for distal lesions had been debated, and the traditional view that total gastrectomy (TG) is required for all gastric lesions has been challenged. Gouzi *et al*[5] conducted a multicenter randomized trial comparing TG to subtotal gastrectomy (SG) enrolling 169 patients with resectable lesions of the gastric antrum. Patients with macroscopic lymph node involvement of the cardioesophageal or splenopancreatic region were excluded from the study. TG consisted of TG with Roux-en-Y esophagojejunostomy. Splenectomy was not routinely performed; however, the TG group had an unspecified higher splenectomy rate than the SG group. SG consisted of a distal gastrectomy and Billroth II gastrojejunostomy. Both procedures included a total omentectomy, and lymph node dissection extended to the pyloric, left gastric, hepatic, and cardiac nodes. The groups were well matched for tumor size, extent of invasion, and lymph node stage. There was a post-operative mortality of 2.4%, with three deaths in the SG group and one death in the TG group. The non-lethal complication rate was 33% in the TG group and 34% in the SG group. Lymph node involvement and serosal extension were significantly associated with 5-year survival; however, extent of resection was not. The authors concluded that TG or SG could be performed with equal morbidity and mortality, but that TG offered no added survival benefit. A major criticism of this study was that it was underpowered to detect a difference between the groups, as 200 patients were originally calculated to show a difference of 20%.

In a larger trial, Bozzetti *et al*[6] also performed a randomized trial comparing TG to SG in 618 patients with resectable tumors at least 6 cm from the cardia. Patients were enrolled if they had tumors of the distal half of the stomach without evidence of metastatic disease, were under 75 years of age and in good health, and had no previous gastric resection or chemotherapy. Patients were further assessed at laparotomy for a minimum distance from the tumor to the cardia and no evidence of peritoneal, D3 lymph node metastasis or extension into adjacent organs. Patients were randomized intra-operatively to TG or SG. Both operations were performed with a D2 lymphadenectomy. Splenectomy was left to the discretion of the operating surgeon. Patients were then followed regularly for a median follow-up of 72 mo in the SG group and 75 mo in the TG group. Five-year survival was 65.3% in the SG, and 62.5% in the TG group, suggesting that type of resection had no influence on the survival. Site of tumor, wall invasion, extension of surgery including splenectomy, and relative frequency of metastatic lymph nodes were significant predictors of survival. Given the lack of survival benefit, the authors concluded that SG was the preferred operation for distal gastric cancer, provided that a proximal margin of at least 6 cm could be obtained, because it is technically less demanding, results in a lower splenectomy rate, and is associated with better quality of life (QOL).

QOL is a very important outcome after gastrectomy. For tumors of the distal stomach, given the current data suggesting equivalent morbidity and survival for TG and SG, the procedure with the best QOL profile is preferred. Davies *et al*[7] compared the QOL of patients following TG to those after SG. These authors compared 46 patients who had undergone an R0 resection. Twenty-six underwent TG and 21 underwent SG. Five questionnaires were used: the Rotterdam symptoms checklist, the Troidl index, the hospital anxiety and depression scale, the activities of daily living score, and the Visick grade. Patients were questioned before the operation and at 1, 3, 6, and 12 mo. There was no difference in QOL prior to the operation or at 1, 3 or 6 mo; however, patients undergoing SG had a significantly higher QOL at 1 year. The authors thus concluded that, given equivalent survival, SG is the operation of choice for tumors of the distal stomach.

Based on data that accrued in prospective, randomized

trials, it is now accepted that SG is an appropriate oncologic operation for distal adenocarcinoma.

Proximal gastric adenocarcinoma

The extent of resection needed to achieve cure in tumors of the gastroesophageal junctions has been a topic of much debate. Ito *et al*[8] reviewed patients with Siewert type II or III carcinoma of the gastric cardia in an attempt to discern the optimal surgical approach. Eighty-two patients were included in the study, 59 with type II and 23 with type III lesions. The surgical approach varied, with 33% undergoing total esophagectomy, 29% undergoing extended gastrectomy with thoracotomy, and 38% undergoing extended gastrectomy without thoracotomy. There was no significant difference in the type of procedure between type II and III lesions. There was no significant difference in post-operative mortality; however, there was a higher post-operative morbidity associated with total esophagectomy as compared to extended gastrectomy with or without thoracotomy (33% *vs* 11%). The addition of a thoracotomy to extended gastrectomy did not have an impact on post-operative morbidity (13% *vs* 10%). There was a significantly higher incidence of microscopic residual disease at the proximal margin in the extended gastrectomy group with or without thoracotomy as compared to the total esophagectomy group (38% *vs* 7%). The mean follow-up was 34 mo and 5-year survival for the entire group was 33%. Multivariate analysis revealed that patient's age over 65, lymph node metastases, and absence of an R0 resection were adversely associated with survival. There was no survival difference between the different types of resection despite a higher incidence of positive proximal margins in the extended gastrectomy group. Based on these results, showing R0 status and nodal status to be predictors of survival, the authors made the following recommendations: (1) A minimum proximal margin of 6 cm and distal margin of 4 cm should be obtained. (2) A minimum of 15 lymph nodes should be sampled. The type of surgical approach should be tailored to fit the individual patient with these goals in mind.

TG is the traditional treatment for proximal gastric cancer; however, this has been recently challenged as well. Harrison *et al*[9] reviewed 98 patients with proximal gastric cancer who underwent gastric resection via an abdominal approach, excluding all patients who underwent esophagogastrectomy. Of these 98 patients, 65 underwent proximal gastrectomy (PG), and 33 underwent TG. There was no difference in post-operative mortality (6% *vs* 3%). Post-operative morbidity data was not analyzed; however, there was no difference in the length of hospital stay. There was no significant difference in time to recurrence or first site of recurrence. The 5-year survival rate was not significantly different between the TG and PG groups with a mean follow-up of 30 mo (43% for PG *vs* 41% for TG). The authors concluded that either procedure can be performed safely without sacrificing long-term survival.

The optimal approach for carcinoma of the gastroesophageal junction or gastric cardia is still a topic of debate. The over-riding factor, regardless of the type of resection, is the ability to obtain a margin-negative resection.

EXTENT OF LYMPH NODE DISSECTION

The extent of lymphadenectomy is one of the most controversial topics in gastric cancer surgery. The Japanese developed an extensive classification system for the regional lymph nodes and a systematic method of dissection referred to now as the D2 resection. This method is described in detail by Maruyama *et al*[10], who also reviewed the Japanese National Cancer Center experience with gastric resection over the period from 1963 to 1985. The authors reported an improvement over this time span in 5-year survival for resected patients from 44.3% to 61.6%. Most of this improvement was attributed to early gastric cancer screening established at a national level; however, they do report an increase in survival over this time period with respects to specific stage, T stage, and N stage.

Shiu *et al*[11] reviewed the results of gastric resection and lymphadenectomy for gastric cancer at Memorial Sloan Kettering Cancer Center over a 20-year period. They analyzed the outcomes of 210 patients and attempted to identify risk factors for mortality. On multivariate analysis, they identified non-pyloric tumor site and greater than three positive lymph nodes as pathologic risk factors. They also identified positive microscopic margins, inadequate lymphadenectomy, and TG as independent surgical risk factors for mortality. Adequate lymphadenectomy was defined as one and a half lymph node levels beyond the furthest level of lymph node disease. For example, patients with N0 disease would require a dissection including the N1 and part of N2 nodes.

This data from a Western center as well as the Japanese data were sited to justify extended lymphadenectomy, a practice which was not commonplace in the West. Multiple clinical trials were performed to look at the morbidity, mortality, and long term survival of a D2 dissection in Western centers.

The best known trial to evaluate lymphadenectomy was from the Dutch Gastric Cancer Group. Bonenkamp *et al*[12] published their results from a randomized controlled trial comparing D1 and D2 gastrectomy in 80 Dutch hospitals over 5 years. D1 resection was defined as containing only the N1 (perigastric) nodes. D2 resection was defined as encompassing the N2 nodes. The spleen and distal pancreas were resected in 11 (3%) of D1 resections and 30 (37%) of D2 resections. The type of gastrectomy, TG *vs* SG, as well as the method of reconstruction were left to the preference of the surgeon. Surgeons were instructed in the technique with video classes, and eight visiting Japanese surgeons were present to supervise all D2 resections. Seven hundred and seven patients underwent resection with intention to treat, and 632 were found to have an R0 resection, and thus followed for evidence of recurrence. These authors reported a statistically significant difference in post-operative mortality (4% *vs* 10%) and complication rate (25% *vs* 43%) for D1 *vs* D2 resection. The 5-year survival rate was 45% for the D1 group and 47% for the D2 group, which was not statistically significant. The 5-year risk of relapse was 43% in the D1 group and 37% in the D2 group, which also was not statistically significant. Subgroup analysis, however, showed that in patients who

did not require splenectomy or pancreatectomy, there was a significantly lower 5-year risk of relapse. The conclusions drawn from this article were that D2 lymphadenectomy was associated with a higher morbidity and mortality without offering a long-term survival benefit, and thus it was not recommended. The sub-group analysis also questioned the benefit of distal pancreatectomy or splenectomy.

A concomitant British trial was also performed over the same time period. Cuschieri *et al*[13] reported the results of a randomized study comparing D1 to D2 resections. Four hundred patients were randomized. In this trial a D1 resection consisted of the removal of all lymph nodes within 3 cm of the tumor and D2 resection consisted of the standard resection of the omental bursa, the hepatoduodenal nodes for antral lesions and the splenic artery, splenic hilar, and retropancreatic nodes by distal pancreatectomy for middle and upper third lesions. They reported similar post operative morbidity and mortality data to that of the Dutch study for the D2 resection group, and showed no difference in the 5-year survival (35% *vs* 33%) or risk of recurrence. As in the Dutch trial, the British trial showed that resection of the spleen and pancreas was independently associated with decreased survival.

Both of these large randomized studies showed no benefit from a D2 resection; however, each also raises the question: is there a role of D2 resection without pancreatico-splenectomy? Two current studies attempt to answer that question.

Edwards *et al*[14] compared D1 resection to D2 resection sparing the pancreas and spleen in a total of 118 patients. The study was not randomized, as one surgeon performed all the D1 resections ($n = 36$) at one hospital and the other surgeon performed all the D2 resections ($n = 82$) at another hospital in the same region of Wales. Splenectomy and pancreatectomy were performed only for evidence of direct invasion of tumor. They reported similar operative mortality (8.3% *vs* 7.3%) and a significant 5-year survival advantage for the D2 resection group (32% *vs* 59%), which was most evident in the patients with stage III disease (8% *vs* 33%). Extent of lymphadenectomy was independently associated with increased survival on multivariate analysis. The authors concluded that a modified D2 resection sparing resection of the pancreas and spleen offers a survival advantage over a D1 resection with no increase in short-term morbidity or mortality.

Recent phase II data from a multicenter trial conducted in Italy showed acceptable morbidity and mortality with good 5-year survival following pancreas-preserving D2 resection[15]. The Italian Gastric Cancer Study Group enrolled 191 patients, and pancreas-preserving D2 resection was performed with an operative mortality of 3.1%, morbidity of 20.9%, and 5-year survival rate of 55%. These numbers compare favorably to the Japanese experience, and suggest that pancreas-preserving D2 may offer a survival benefit; however, the randomized comparison to D1 resection has not yet been performed.

Data from Japan concerning mortality and 5-year survival after gastrectomy had been criticized for being retrospective in nature. A D1 gastrectomy is considered as a non-curative operation in Japan; thus, it would not be performed in any randomized trial. Sano *et al*[16] are conducting a randomized trial comparing standard D2 resection to extended para-aortic lymphadenectomy. This is the first randomized prospective study to come from Japan. The selection criteria of the surgeons and the selected centers should be mentioned. Only surgeons with over 100 D2 gastrectomies, and only centers with over 80 gastrectomies a year were selected for the trial. This is in stark contrast to the Dutch and British trials which did not require a set training period to master the "learning curve" of the procedure. The spleen was resected in all the cases where TG was performed, and the pancreas was resected only in cases of direct invasion. The morbidity and mortality data for this trial have been released. The overall morbidity of a D2 resection was 20.9%, with a mortality of 0.8%. The conclusions that can be drawn from this study, pending the 5-year survival data, are that D2 resection can be performed at specialized centers by surgeons with ample experience with low morbidity and mortality.

Based on the current data, it appears that a modified D2 lymphadenectomy, sparing the spleen and pancreas when possible, can be performed safely and may offer the best chance for long-term survival; however, the randomized controlled data to support this argument is currently in progress.

EXTENDING RESECTION TO ADJACENT ORGANS

D2 resection with splenectomy and distal pancreatectomy was performed by many centers in Japan to facilitate dissection of lymph nodes around the splenic artery with early reports suggesting improved survival[10]. The utility of extended resection was later called into question by many Japanese and Western surgeons. Otsuji *et al*[17] reviewed 128 patients who underwent TG for gastric adenocarcinoma of the middle or proximal stomach. Of these, 35.9% underwent pancreaticosplenectomy, 44.6% splenectomy, and 19.5% gastrectomy alone. Pancreaticosplenectomy increased the risk of pancreatic fistula significantly. Five-year survival for the pancreaticosplenectomy group, the splenectomy group and the gastrectomy alone group were 40.7%, 55.9%, and 54.2%, respectively; however, on multivariate analysis pancreaticosplenectomy and splenectomy alone were not independently associated with survival. The conclusions drawn from this study were that extension of TG to include pancreaticosplenectomy or splenectomy increases the risk of complications without improving survival.

Kasakura *et al*[18] reviewed 1 938 gastric resections over 18 years and also concluded that splenectomy and distal pancreatectomy do not have an impact on survival and are associated with an increased incidence of complications. Of these 1 938 patients, 78 underwent splenectomy (S), 105 underwent splenectomy/pancreatectomy (PS), and 1 755 underwent gastrectomy alone. The PS and S groups were associated with a higher percentage of proximal tumors and TG, higher T stage, higher N stage, and worse histologic grade. There were more severe post-operative

complications in the PS and S groups over the gastrectomy alone group, with a higher rate of pancreatic fistula, intra-abdominal abscess, and anastomotic leak. There was also a higher rate of local recurrence in the S and PS groups. When analyzed by UICC stage; however, no difference in 5-year survival for stage II, III, or IV tumors was detected. Gastrectomy alone was found to have higher 5-year survival for T2 tumors; however, for T3 and T4 tumors, there was no difference. Conclusions from this study were that long-term survival is not affected by splenectomy or pancreatectomy and these procedures should not be performed solely to aid in lymph node dissection. The spleen should only be resected when there are clearly positive lymph nodes in the splenic hilum and around the splenic artery, and the pancreas should only be resected when there is direct invasion of tumor.

Resection of adjacent organs for local invasion was advocated in the early Japanese literature[10]; however, this practice also has been analyzed extensively in the past decade. Shchepotin *et al*[19] reviewed 353 patients with T4 gastric cancer who underwent gastrectomy combined with resection of adjacent organs. Of these patients, 89% had histologically confirmed invasion. TG was performed in 32.9% of patients and SG in 67.1%. The extent of lymph node dissection was the same in all patients, consisting of dissection of all perigastric nodes, nodes along the celiac axis, hepatic artery, and proximal splenic artery. The transverse colon was resected in 45%, the tail of the pancreas and spleen in 42.5%, the left hepatic lobe in 28.5% and the head of the pancreas in 10.5%. The complication rate was 31.2%, and mortality rate was 13.6%. Combined 5-year survival was 25%; however, when this was broken down into node positive or node negative T4 lesions, 5-year survival changed significantly. Node negative T4 patients had a 37% 5-year survival, whereas node positive T4 patients had only a 15% survival. From these data, the authors continued to advocate extended resection of adjacent organs in the resection of gastric cancer for cure.

Martin *et al*[20] showed that extended organ resection could be performed with acceptable morbidity (4%) and 5-year survival (32%). Two hundred and sixty-eight patients with locally advanced gastric cancer underwent gastrectomy with adjacent organ resection and D2 lymphadenectomy. The type of gastrectomy was variable; however, there was a higher incidence of adjacent organ resection in the TG group. Spleen alone was the most common resection ($n=123$), followed by spleen/pancreas ($n=38$), spleen/colon ($n=18$), pancreas alone ($n=12$), and colon alone ($n=16$). As would be expected, there was a higher percentage of T3/T4 tumors and N2/N3 lymph node metastases in the organ resection group. Perioperative mortality was not significantly different, 3.6% in the gastrectomy group and 3.7% in the gastrectomy with organ resection group. There was a higher rate of recurrence in the gastrectomy with organ resection group (52% *vs* 42% at 24 mo follow-up), and significantly lower median survival (32 mo *vs* 63 mo) on univariate analysis. On multivariate analysis, however, only T-stage, N-stage and overall stage were predictors of survival. The authors concluded that resection of adjacent organs is important to achieve an R0 resection and can be done with minimal morbidity; however, careful selection of clinically T4 tumors should be performed to limit unnecessary organ resection in early stage gastric cancer.

Resection of adjacent organs in conjunction with gastrectomy can increase survival with minimal additional morbidity in a highly selected patient population.

POSITIVE MARGINS

Patients with recurrence after resection of gastric cancer uniformly have a rapid decline. The risk factors for recurrence, and the rate and pattern of recurrence has been examined. In a recent retrospective review of 367 patients who underwent an R0 resection with subsequent recurrence, the rate and pattern of recurrence was examined[21]. The majority of the patients (65%) had T3 lesions, 46% had N1 nodal disease, and 54% were AJCC stage III. The type of gastric resection was highly variable, with the highest percent being esophago-proximal resections (45%). The extent of lymphadenectomy was also variable, with the majority being D2 (68%). The rate of locoregional, peritoneal, and distant metastases was 54%, 51%, and 29% respectively, with significant overlap of these three. Seventy-nine percent had recurred within two years and 94% by four years. The median time to death from diagnosis of recurrence was 6 mo. The pattern of recurrence was not associated with survival time; however, T stage, N stage, age and presentation with symptoms were all predictors of decreased survival. Advanced T stage, distal location, diffuse Lauren's subtype, and female gender were significantly associated with peritoneal metastases. Proximal location and male gender were associated with locoregional recurrence. Proximal location, early T stage, and intestinal Lauren's subtype were associated with distal recurrence. There was no significant pattern of recurrence associated with overall stage, extent of lymphadenectomy, nodal status, or extension of resection to adjacent organs.

There is no surgical therapy for recurrent gastric cancer; however, the question of re-excision for positive margins at primary resection has been raised. Survival for patients with microscopically positive margins has been shown to be significantly shorter than those with clear margins[25]. In a recent review of 259 patients who underwent gastrectomy with curative intent, 22 had microscopically positive resection line margins[22]. Positive margins were associated with tumor location and differentiation. There was a significantly lower 5-year survival for the patients with positive margins (18% *vs* 45%); however, when this was stratified for lymph node status, the patients with positive lymph nodes had a significantly lower survival. The authors concluded that, given the very poor prognosis associated with positive margins, re-laparotomy may be justified in those patients with node-negative disease.

PALLIATIVE SURGERY FOR GASTRIC CANCER

Despite improved clinical outcomes associated with earlier diagnosis, more accurate staging, and decreased surgical morbidity and mortality, the overall prognosis of gastric

cancer remains poor because many patients are incurable at presentation. A complete R0 resection remains the most powerful indicator of survival, but is obtained in only 50% of those presenting for resection of a primary gastric cancer[20,23]. For those patients who present with stage IV disease, cure measured by 5-year survival is exceedingly rare and is not a realistic treatment goal[2,24-32]. Because of the low cure rate and the advanced stage at which many patients present, palliative strategies and symptom management remain an essential component in the total care of the patient with gastric cancer[33].

Palliation of symptoms caused by any advanced cancer demands the highest level of surgical judgment. Although an important part of the surgical decision making process requires consideration of risk in terms of treatment-related toxicity, morbidity, and mortality, attention to this element should not be the predominant factor in making decisions about palliative therapy. Decisions are made best on endpoints such as the probability and durability of symptom resolution, the impact on overall QOL, and pain control. Deliberations must consider the medical condition and performance status of the patient, the extent and prognosis of the cancer, the availability and success of non-surgical management, cost effectiveness and the individual patient's quality and expectancy of life. Knowledge about the need to repeat a specific therapy or requirements to manage additional symptoms can give further information about the potential for symptom-free survival and what additional care will be needed. Therapy for symptoms must remain flexible and individualized to continually meet the patient's unique and ever changing needs[34-36]. Surgical palliation of advanced gastric cancer may include resection or bypass, alone or in combination with endoscopic or percutaneous interventions. Such interventions have been proposed not only to improve symptom control, but also to eliminate potential complications (bleeding, obstruction, pain, perforation, and debilitating ascites) caused by the primary tumor[37,38].

In 1958, Lawrence and McNeer demonstrated that palliative gastric resections effectively relieve symptoms in patients with incurable gastric cancer. Because of high rates of associated perioperative morbidity and mortality and the brief period of anticipated survival, however, the authors suggested that a TG in patients with incurable gastric cancer was rarely a worthwhile palliative procedure[39]. This conclusion was supported by Remine in 1979 who also suggested that TG was not a satisfactory palliative operation[40]. Later series, however, showed improved symptom relief with gastrectomy compared to gastroenterostomy, without increasing complication rates[41,42]. Others have based their support for palliative gastric resections primarily on improved survival data and have proposed that it should be performed whenever technically possible[43,44]. Because of decreasing perioperative complications, some authors now suggest that total palliative gastrectomy and esophagogastrectomy is justified in selected patients[45-49].

The effective and appropriate use of palliative surgical interventions in patients with gastric cancer remains controversial. Recommendations from the literature are contradictory and often based on the retrospective evaluation of suboptimal data. A highly variable and imprecise understanding of the goals and indications of palliative surgery, poorly defined patient groups, and a reliance on inappropriate endpoints contributes to this confusion. The designation of patients as "palliative" is commonly based on the extent of disease (ranging from gross disease at operation to postoperative margin status) rather than a sound definition encompassing factors associated with good palliative therapy. Even though the value of a palliative procedure should be judged by its ability to control symptoms, reports often fail to utilize validated QOL or pain assessment instruments and rarely consider the durability of potential palliative benefits[25]. These factors limit useful interpretation of most prior studies on palliative procedures for gastric cancer. The impact of such deficiencies in the literature has been demonstrated by a recent analysis of the Memorial Sloan-Kettering Gastric Cancer Database. Important differences among the patients undergoing a non-curative gastric cancer were demonstrated when a sound and reproducible definition of palliative surgery was applied. Significant differences between overall survival, primary tumor sites, staging, degrees of nodal and metastatic disease, and the types of procedures performed support the differentiation between palliative and non-palliative designations[49].

Conclusions over the effectiveness of palliative operations in the gastric cancer literature are often based, incorrectly, on incremental survival differences. Caution must be taken when evaluating survival data in patients following a palliative intervention. Palliative care ideally selects treatment that will maximize QOL and minimize complications. Consideration of anticipated survival helps to define a period during which the requirements of effective symptom control must be met and may be useful when considering the risk-benefit ratio for an individual patient[50,51]. Although increased survival may be a secondary goal of a palliative procedure, it is inappropriate to select a palliative procedure solely based on improved duration of survival[36]. Based on patients grouped by extent of disease rather than palliative intent, The Dutch Gastric Cancer Group has suggested that differences in overall survival following "palliative" gastric resections may be beneficial in patients with tumor load restricted to one metastatic site[52]. Such conclusions about the value of palliative gastrectomy are premature because they fail to consider the associated risks, benefits, and expected durability of the procedure[33,49].

To compare the impact of potentially achievable gains associated with palliative surgery, non-curative gastrectomies were analyzed with a partitioned survival analysis[33,53]. The technique, which has been generally used to evaluate chemotherapy trials, analyzes treatment by defining relevant clinical health states and comparing their duration with regard to treatment, toxicity and relapse. The analysis provided by this methodology is well suited to evaluate surgical palliation, especially in conditions such as gastric cancer, in which treatment-related toxicity plays such an important role in both the literature and in clinical decision-making[33,54]. Palliative gastric resections previously have been associated with considerable operative morbidity (54%) and mortality (6%)[49]. Duration in the time without

symptoms or toxicity (TWiST) state was found to be significantly decreased (8.5 mo *vs* 2.1 mo, $P = 0.04$) in patients experiencing a major postoperative complication (requiring invasive interventions, unplanned ICU admission, or permanent disability). Complications have a considerable influence on the patients' limited survival by not only increasing time in a hospitalized setting but also by diminishing the QOL. Concerns about potential complications such as bleeding, obstruction or perforation have led some to propose prophylactic gastric resections in patients with advanced, asymptomatic gastric cancer. Sarela *et al* have shown in patients with known metastatic disease identified on laparoscopy; however, that complications requiring emergency or urgent palliative operations were rare in patients not receiving a prophylactic resection for incurable gastric cancer[55].

Since palliative gastrectomies are associated with significant perioperative morbidity and mortality, the authors recommend deliberate palliative resection only in carefully selected patients with severe symptoms[34,50,54,55].

REFERENCES

1 **Stell DA**, Carter CR, Stewart I, Anderson JR. Prospective comparison of laparoscopy, ultrasonography and computed tomography in the staging of gastric cancer. *Br J Surg* 1996; **83**: 1260-1262
2 **Burke EC**, Karpeh MS, Conlon KC, Brennan MF. Laparoscopy in the management of gastric adenocarcinoma. *Ann Surg* 1997; **225**: 262-267
3 **Hulscher JB**, Nieveen van Dijkum EJ, de Wit LT, van Delden OM, van Lanschot JJ, Obertop H, Gouma DJ. Laparoscopy and laparoscopic ultrasonography in staging carcinoma of the gastric cardia. *Eur J Surg* 2000; **166**: 862-865
4 **Bentrem D**, Wilton A, Mazumdar M, Brennan M, Coit D. The value of peritoneal cytology as a preoperative predictor in patients with gastric carcinoma undergoing a curative resection. *Ann Surg Oncol* 2005; **12**: 347-353
5 **Gouzi JL**, Huguier M, Fagniez PL, Launois B, Flamant Y, Lacaine F, Paquet JC, Hay JM. Total versus subtotal gastrectomy for adenocarcinoma of the gastric antrum. A French prospective controlled study. *Ann Surg* 1989; **209**: 162-166
6 **Bozzetti F**, Marubini E, Bonfanti G, Miceli R, Piano C, Gennari L. Subtotal versus total gastrectomy for gastric cancer: five-year survival rates in a multicenter randomized Italian trial. Italian Gastrointestinal Tumor Study Group. *Ann Surg* 1999; **230**: 170-178
7 **Davies J**, Johnston D, Sue-Ling H, Young S, May J, Griffith J, Miller G, Martin I. Total or subtotal gastrectomy for gastric carcinoma? A study of quality of life. *World J Surg* 1998; **22**: 1048-1055
8 **Ito H**, Clancy TE, Osteen RT, Swanson RS, Bueno R, Sugarbaker DJ, Ashley SW, Zinner MJ, Whang EE. Adenocarcinoma of the gastric cardia: what is the optimal surgical approach? *J Am Coll Surg* 2004; **199**: 880-886
9 **Harrison LE**, Karpeh MS, Brennan MF. Total gastrectomy is not necessary for proximal gastric cancer. *Surgery* 1998; **123**: 127-130
10 **Maruyama K**, Okabayashi K, Kinoshita T. Progress in gastric cancer surgery in Japan and its limits of radicality. *World J Surg* 1987; **11**: 418-425
11 **Shiu MH**, Moore E, Sanders M, Huvos A, Freedman B, Goodbold J, Chaiyaphruk S, Wesdorp R, Brennan MF. Influence of the extent of resection on survival after curative treatment of gastric carcinoma. A retrospective multivariate analysis. *Arch Surg* 1987; **122**: 1347-1351
12 **Bonenkamp JJ**, Hermans J, Sasako M, van de Velde CJ, Welvaart K, Songun I, Meyer S, Plukker JT, Van Elk P, Obertop H, Gouma DJ, van Lanschot JJ, Taat CW, de Graaf PW, von Meyenfeldt MF, Tilanus H. Extended lymph-node dissection for gastric cancer. *N Engl J Med* 1999; **340**: 908-914
13 **Cuschieri A**, Weeden S, Fielding J, Bancewicz J, Craven J, Joypaul V, Sydes M, Fayers P. Patient survival after D1 and D2 resections for gastric cancer: long-term results of the MRC randomized surgical trial. Surgical Co-operative Group. *Br J Cancer* 1999; **79**: 1522-1530
14 **Edwards P**, Blackshaw GR, Lewis WG, Barry JD, Allison MC, Jones DR. Prospective comparison of D1 vs modified D2 gastrectomy for carcinoma. *Br J Cancer* 2004; **90**: 1888-1892
15 **Degiuli M**, Sasako M, Ponti A, Calvo F. Survival results of a multicentre phase II study to evaluate D2 gastrectomy for gastric cancer. *Br J Cancer* 2004; **90**: 1727-1732
16 **Sano T**, Sasako M, Yamamoto S, Nashimoto A, Kurita A, Hiratsuka M, Tsujinaka T, Kinoshita T, Arai K, Yamamura Y, Okajima K. Gastric cancer surgery: morbidity and mortality results from a prospective randomized controlled trial comparing D2 and extended para-aortic lymphadenectomy--Japan Clinical Oncology Group study 9501. *J Clin Oncol* 2004; **22**: 2767-2773
17 **Otsuji E**, Yamaguchi T, Sawai K, Okamoto K, Takahashi T. Total gastrectomy with simultaneous pancreaticosplenectomy or splenectomy in patients with advanced gastric carcinoma. *Br J Cancer* 1999; **79**: 1789-1793
18 **Kasakura Y**, Fujii M, Mochizuki F, Kochi M, Kaiga T. Is there a benefit of pancreaticosplenectomy with gastrectomy for advanced gastric cancer? *Am J Surg* 2000; **179**: 237-242
19 **Shchepotin IB**, Chorny VA, Nauta RJ, Shabahang M, Buras RR, Evans SR. Extended surgical resection in T4 gastric cancer. *Am J Surg* 1998; **175**: 123-126
20 **Martin RC**, Jaques DP, Brennan MF, Karpeh M. Extended local resection for advanced gastric cancer: increased survival versus increased morbidity. *Ann Surg* 2002; **236**: 159-165
21 **D'Angelica M**, Gonen M, Brennan MF, Turnbull AD, Bains M, Karpeh MS. Patterns of initial recurrence in completely resected gastric adenocarcinoma. *Ann Surg* 2004; 240: 808-816
22 **Wanebo HJ**, Kennedy BJ, Chmiel J, Steele G, Winchester D, Osteen R. Cancer of the stomach. A patient care study by the American College of Surgeons. *Ann Surg* 1993; **218**: 583-592
23 **Cascinu S**, Giordani P, Catalano V, Agostinelli R, Catalano G. Resection-line involvement in gastric cancer patients undergoing curative resections: implications for clinical management. *Jpn J Clin Oncol* 1999; **29**: 291-293
24 **Kooby DA**, Coit DG. Controversies in the surgical management of gastric cancer. *J Natl Compr Canc Netw* 2003; **1**: 115-124
25 **Smith JW**, Brennan MF. Surgical treatment of gastric cancer. Proximal, mid, and distal stomach. *Surg Clin North Am* 1992; **72**: 381-399
26 **Allum WH**, Fielding JW. Gastric cancer: a 25-year review. *Br J Surg* 1990; **77**: 353
27 **Breaux JR**, Bringaze W, Chappuis C, Cohn I. Adenocarcinoma of the stomach: a review of 35 years and 1,710 cases. *World J Surg* 1990; **14**: 580-586
28 **Thompson GB**, van Heerden JA, Sarr MG. Adenocarcinoma of the stomach: are we making progress? *Lancet* 1993; **342**: 713-718
29 **Neugut AI**, Hayek M, Howe G. Epidemiology of gastric cancer. *Semin Oncol* 1996; **23**: 281-291
30 **Hundahl SA**, Menck HR, Mansour EG, Winchester DP. The National Cancer Data Base report on gastric carcinoma. *Cancer* 1997; **80**: 2333-2341
31 **Davies J**, Chalmers AG, Sue-Ling HM, May J, Miller GV, Martin IG, Johnston D. Spiral computed tomography and operative staging of gastric carcinoma: a comparison with histopathological staging. *Gut* 1997; **41**: 314-319
32 **Miller FH**, Kochman ML, Talamonti MS, Ghahremani GG, Gore RM. Gastric cancer. Radiologic staging. *Radiol Clin North Am* 1997; **35**: 331-349
33 **Miner TJ**, Karpeh MS. Gastrectomy for gastric cancer: defining critical elements of patient selection and outcome assessment. *Surg Oncol Clin N Am* 2004; **13**: 455-66, viii
34 **Miner TJ**, Brennan MF, Jaques DP. A prospective, symptom

related, outcomes analysis of 1022 palliative procedures for advanced cancer. *Ann Surg* 2004; **240**: 719-26; discussion 726-7
35 **Miner TJ**, Jaques DP, Shriver CD. A prospective evaluation of patients undergoing surgery for the palliation of an advanced malignancy. *Ann Surg Oncol* 2002; **9**: 696-703
36 **Miner TJ**, Jaques DP, Tavaf-Motamen H, Shriver CD. Decision making on surgical palliation based on patient outcome data. *Am J Surg* 1999; **177**: 150-154
37 Abdalla EK, Kaw M, Pisters PW. Invasive techniques for palliation of advanced gastric cancer. In: Posner MC, Vokes EE, Weichselbaum RR, eds. American Cancer Society Atlas of Clinical Oncology: Cancer of the upper gastrointestinal tract. Hamilton, Ontario: BC Decker Inc; 2002: 308-321
38 **Sugarbaker PH**, Yonemura Y. Clinical pathway for the management of resectable gastric cancer with peritoneal seeding: best palliation with a ray of hope for cure. *Oncology* 2000; **58**: 96-107
39 **Lawrence W**, Mcneer G. The effectiveness of surgery for palliation of incurable gastric cancer. *Cancer* 1958; **11**: 28-32
40 **ReMine WH**. Palliative operations for incurable gastric cancer. *World J Surg* 1979; **3**: 721-729
41 **Meijer S**, De Bakker OJ, Hoitsma HF. Palliative resection in gastric cancer. *J Surg Oncol* 1983; **23**: 77-80
42 **Geoghegan JG**, Keane TE, Rosenberg IL. Gastric cancer : the case for a selectively policy in surgical management. *J R Coll Surg Edinb* 1993; **38**: 1863-1868
43 **Bozzetti F**, Bonfanti G, Audisio RA, Doci R, Dossena G, Gennari L, Andreola S. Prognosis of patients after palliative surgical procedures for carcinoma of the stomach. *Surg Gynecol Obstet* 1987; **164**: 151-154
44 **Ouchi K**, Sugawara T, Ono H, Fujiya T, Kamiyama Y, Kakugawa Y, Mikuni J, Yamanami H. Therapeutic significance of palliative operations for gastric cancer for survival and quality of life. *J Surg Oncol* 1998; **69**: 41-44
45 **Welvaart K**, de Jong PL. Palliation of patients with carcinoma of the lower esophagus and cardia: the question of quality of life. *J Surg Oncol* 1986; **32**: 197-199
46 **Monson JR**, Donohue JH, McIlrath DC, Farnell MB, Ilstrup DM. Total gastrectomy for advanced cancer. A worthwhile palliative procedure. *Cancer* 1991; **68**: 1863-1868
47 **Boddie AW**, McMurtrey MJ, Giacco GG, McBride CM. Palliative total gastrectomy and esophagogastrectomy. A reevaluation. *Cancer* 1983; **51**: 1195-1200
48 **Ekbom GA**, Gleysteen JJ. Gastric malignancy: resection for palliation. *Surgery* 1980; **88**: 476-481
49 **Miner TJ**, Jaques DP, Karpeh MS, Brennan MF. Defining palliative surgery in patients receiving noncurative resections for gastric cancer. *J Am Coll Surg* 2004; **198**: 1013-1021
50 **Miner TJ**, Jaques DP, Paty PB, Guillem JG, Wong WD. Symptom control in patients with locally recurrent rectal cancer. *Ann Surg Oncol* 2003; **10**: 72-79
51 **Forbes JF**. Principles and potential of palliative surgery in patients with advanced cancer. *Recent Results Cancer Res* 1988; **108**: 134-142
52 **Hartgrink HH**, Putter H, Klein Kranenbarg E, Bonenkamp JJ, van de Velde CJ. Value of palliative resection in gastric cancer. *Br J Surg* 2002; **89**: 1438-1443
53 **Miner TJ**, Jaques DP, Karpeh MS, Brennan MF. Exploring the value of non-curative gastric resections performed with palliative intent. *Proc Am Soc Clin Oncol* 2003; **22**: 266
54 **Ball AB**, Baum M, Breach NM. Surgical Palliation. In: Derek D, Hanks GWC, MacDonald N, Eds. Oxford Textbook of Palliative Medicine. Oxford, England: Oxford Press; 1998: 282-297
55 **Sarela AI**, Miner TJ, Jaques DP, Karpeh MS, and Brennan MF. Outcomes for patients with laparoscopically staged M1 gastric adenocarcinoma. *Br J Surg 2004*; **91**: 514-520

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REVIEW

Gene therapy for gastric cancer: Is it promising?

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Abstract

Gastric cancer is one of the most common tumors worldwide. The therapeutic outcome of conventional therapies is inefficient. Thus, new therapeutic strategies are urgently needed. Gene therapy is a promising molecular alternative in the treatment of gastric cancer, including the replacement of defective tumor suppressor genes, the inactivation of oncogenes, the introduction of suicide genes, genetic immunotherapy, anti-angiogenetic gene therapy, and virotherapy. Improved molecular biological techniques and a better understanding of gastric carcinogenesis have allowed us to validate a variety of genes as molecular targets for gene therapy. This review provides an update of the new developments in cancer gene therapy, new principles, techniques, strategies and vector systems, and shows how they may be applied in the treatment of gastric cancer.

Key words: Gene therapy; Gastric cancer; Virotherapy

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INTRODUCTION

Gastric cancer is the fourth most common malignancy worldwide with an estimated 934 000 new cases that was reported in 2002 and the second most common cause of death from cancer (700 000 deaths annually). Almost two-thirds of the cases occur in developing countries and 42% in China alone[1]. The prognosis of gastric cancer is poor with an estimated relative 5-year survival rate of less than 20%[2]. The efficacy of current therapeutic approaches such as surgery, hormone, radio- and chemotherapy is limited. Thus, new therapeutic approaches are urgently needed. Cancer is an acquired genetic disease developing in a multi-step process. Mutations of genes related to growth control, apoptosis, invasion and metastasis form the molecular genetic basis of malignant transformation and tumor progression[3]. The characterization of dysregulated genes like the tumor suppressor p53, which are critical for carcinogenesis, and a better understanding of the molecular basis for tumor-host interaction led to significant progress in the development of new therapeutic agents. More than 15 years ago, gene therapy emerged as a new therapeutic approach and has meanwhile become an important strategy in cancer treatment. Cancer is by far the most frequent of all indications addressed by gene therapy (60% of all clinical trials[3]), underlining the expectations raised by this new therapeutic option. The original concept of cancer gene therapy was further developed into two branches as a result of different strategies of therapeutic benefit: molecular cancer therapy and virotherapy. Molecular cancer therapy can be defined as a therapeutic technique, which aims at the introduction of nucleic acids into cancer patients' cells in order to modulate the gene expression profile of the target cells and thereby eradicate the tumor[4]. In contrast, virotherapy is a new concept of gene therapy that uses replication-competent oncolytic viral vectors (OVV) with viro-oncolytic potency for targeted tumor cell destruction[5].

There are different ways to modulate tumor growth by gene therapeutic strategies. These include direct destruction of tumor cells, inhibition of tumor angiogenesis and tumor cell spread and activation of the host immune response against the tumor. Although all these approaches showed promising anti-tumor effects in pre-clinical investigations, clinical trials have often been disappointing, since they demonstrated only slight therapeutic benefit. Thus, a major breakthrough is still needed in gene therapy of cancer. Nevertheless, clinical trials proved the relative safety of human cancer gene therapy. The application of vectors and the expression of transgenes are generally well tolerated and the low risk of severe side effects seems to be calculable.

The main problem of the relative inefficiency of cancer gene therapy continues to be the low *in vivo* efficiency of gene delivery into the target tumor cells, leading to low expression of therapeutic genes and thus limited curative effects. Several factors seem to be responsible for this, among them the presence of anatomic barriers inhibiting the efficient transfer of vectors from circulation to target cells and the low expression of vector-specific target receptors on the cancer cells causing reduced

cellular uptake of viral vectors. Moreover, immunological responses of the host against the vectors and the rapid clearance of vectors from circulation after intravascular administration may be important factors preventing efficient gene transfer[6-8]. In the last few years, great efforts have been made to overcome these limitations. Especially the development of OVV[9] and vectors with enhanced tumor-specific targeting[10,11] together with improved vector application protocols have led to a significant enhancement of vector-mediated gene delivery. Furthermore, the use of new powerful molecular techniques like RNA interference (RNAi)[12] and the detection of new target genes are hopeful signs for improving human cancer by gene therapy. In the present paper, we review new trends in gene therapy and update their application in gastric cancer.

TUMOR SUPPRESSOR GENES

The most obvious way to target growth regulation in cancer cells is to introduce tumor suppressors that may be inactivated in tumors. The replacement of p53, which is the most commonly inactivated tumor suppressor and mutated in about 60% of human gastric cancers, has emerged as an attractive treatment option, both alone and combined with conventional chemotherapy[13,14]. Introduction of the p53 gene via a recombinant adenovirus has been shown to inhibit the growth of gastric cancer cells with mutated p53 *in vitro* and *in vivo*[15,16]. The pro-apoptotic function of p53 depends on the transactivation of genes such as Bax, Apaf-1, Fas, and PTEN, whose own expression or activity may be abnormal in tumor cells[17]. Consequently, the Bax gene may serve as a good alternative to p53 for cancer gene therapy, not only because it has been shown to kill cancer cells irrespective of their p53 status, but also because it may increase their sensitivity to other anti-tumor treatments[18]. Moreover, the adenoviral expression of the initiator caspase-8 leads to the selective induction of apoptosis in detached gastric tumor cells *in vitro* and *in vivo*, thus displaying anti-metastatic potential in gastric cancer[19]. All these signaling molecules work through a common pathway involving activation of the effector caspase 3. Thus, recombinant expression of caspase 3 leads to the induction of apoptosis in gastric cancer cells[20]. Moreover, the introduction of wild-type p16INK4A, another tumor suppressor and cell cycle regulator in gastric cancer cells harboring a p16 mutation, may also be a feasible approach to efficient tumor growth control and chemosensitization[21]. Finally, the replacement of Fhit, a tumor suppressor often inactivated in gastric cancer, decreases sensitivity to carcinogens and induces apoptosis in gastric tumor cells *in vivo*. Therefore, restoring Fhit expression by viral transduction may be a promising strategy for both the prevention and therapy of gastric tumors[22].

SUICIDE GENES

This strategy relies on the conversion of non-toxic substances (prodrugs) into physiologically active agents by means of non-mammalian enzymes. These suicide enzymes are over-expressed in neoplastic cells as a result of successful transfection with their genes[23]. The most widely used suicide gene/prodrug system is the herpes simplex virus (HSV) thymidine kinase (HSV-tk)/ganciclovir (GCV) system that can convert the prodrug GCV into phosphorylated GCV. The phosphorylated GCV inhibits cellular DNA synthesis and leads to the killing of cancer cells via apoptotic and non-apoptotic mechanisms[24,25]. One of the powerful features in these systems is the "bystander effect", the mechanism by which the toxic metabolites are transferred from transduced cells to neighboring cancer cells via gap junctions or apoptotic vesicles. The bystander effect drastically enhances the tumor-killing capacity of the HSV-tk/GCV system[26,27]. Several studies were undertaken to evaluate the potential of suicide gene therapy in gastric cancer. In alpha-fetoprotein (AFP)-producing gastric tumors, the adenovirus-mediated expression of HSV-tk by an AFP enhancer/promoter element selectively eliminated AFP-positive, but not AFP-negative cell lines when treated with ganciclovir[28]. This approach may be a promising tumor-selective treatment option for AFP-positive gastric tumors with a very poor prognosis. A similar approach involves the expression of recombinant *E. coli* cytosine deaminase (CD) in gastric cancer cells together with the administration of 5-fluorocytosine (5-FC). 5-FC is given orally and converted to 5-fluorouracil in the tumor cells expressing CD. In attempts to increase the specificity of suicide gene therapy, CD expressed from gastric cancer cell-specific promoters SEL1L and TP1 was shown to cause efficient cytotoxic effects in combination with 5-FC[29]. An earlier attempt with tumor-specific and more efficient CD/5-FC gene therapy was carried out using the Cre/loxP regulation system. Ueda *et al*[30] constructed an adenovector-expressing Cre recombinase from a carcinoembryonic antigen (CEA) promoter and a second vector expressing CD under the control of the CAG promoter. The double infection with both vectors rendered CEA-producing gastric cancer cells 13 times more sensitive to 5-FC than the single infection with a vector expressing CD from the CEA promoter. Consequently, anti-tumor efficacy *in vivo* was also significantly enhanced by using the Cre/loxP system compared to the single infection with the vector directly expressing CD under the control of the CEA promoter. Finally, recombinant expression of the bacterial enzyme nitroimidazole reductase gene together with the administration of the prodrug CB1954 was evaluated in a phase I and pharmacokinetic study with the intention of treating gastric cancer[31].

ANTI-ANGIOGENESIS GENE THERAPY

Tumor angiogenesis plays an important role in the growth of solid tumors and the formation of metastases. Angiogenesis is a multi-level process including endothelial cell proliferation, migration, basement membrane degradation, and lumen reorganization. It is stimulated by several factors secreted from both host and tumor cells. The principal growth factors driving angiogenesis include, among others, the vascular endothelial growth factor (VEGF), the basic fibroblast growth factor, and the hepatocyte growth factor (HGF)[32]. Thus, there are various

potential targets for anti-angiogenic cancer gene therapy. In contrast to other genetic treatments, anti-angiogenic gene therapy does not necessarily require direct and selective transduction of target genes into cancer cells, but rather transduction around the tumor to create an anti-angiogenic environment[33]. This advantage helps to overcome the limitations of the currently available vector systems, which often lack adequate transduction efficiency in cancer cells. Several studies were undertaken to evaluate the potential of anti-angiogenic gene therapy in gastric cancer. One study demonstrated that, if expressed from adenovector-transduced peritoneal mesothelial cells, the soluble VEGF receptor sFlt-1 is able to inhibit the peritoneal dissemination of gastric cancer *in vivo* and consequently prolong the survival of treated animals[33]. Another study evaluated the therapeutic efficacy of the HGF antagonist NK4, which is known for its inhibitory effects on several angiogenetic pathways. Application of an NK4-expressing adenovector inhibited the formation of both peritoneal metastases and intra-tumor vessels in gastric cancer *in vivo*[34]. New potential targets for anti-angiogenic gene therapy of gastric cancer were recently discovered. Meng *et al*[35] and Xue *et al*[36] showed that silencing Raf-1 and Rac1 GTPase, which are critical factors in hypoxia-induced gene activation of several angiogenesis factors, results in downregulation of the angiogenesis-promoting factors VEGF and Hif-1α and upregulation of the tumor suppressors and angiogenesis inhibitors p53 and VHL. Furthermore, downregulation of Raf-1 and Rac1 GTPase leads to tumor cell apoptosis and significantly inhibits cell proliferation. Similarly, Stoeltzing *et al*[37] showed that direct suppression of Hif-1α resulted in decreased secretion of VEGF, thereby impairing tumor growth, angiogenesis and vessel maturation *in vivo*.

GENETIC IMMUNOTHERAPY

Genetic immunotherapy aims at improving the host's immune response to a particular tumor and is currently one of the most promising gene therapeutic options for cancer. The function of the immune system is very complex and its activation in gene therapeutic settings can be achieved by employing different strategies[38]. One of the most common strategies in immunotherapy of cancer is the use of mediators of the immune system. Among them, IL-2, IL-12, INF-γ, GM-CSF and TNF-α have raised special attention and several trials have proved their efficacy in cancer gene therapy[39-42]. New developments indicate further improvement of the benefit, if cytokine therapy is combined or used with other gene therapeutic options. For example, synergistic anti-tumor effects were achieved by simultaneous expression of IL-2 and INF-γ[40] or by combining an oncolytic adenovirus (oAdV) with IL-12 immunotherapy[43].

Based on this knowledge, studies were carried out in order to prove the efficacy of immunotherapy in combination with other gene therapeutic strategies in gastric cancer. Zhang *et al*[44-46] evaluated the anti-tumor effects of the HSV-tk/GCV system together with the expression of recombinant IL-2 or TNF-α in gastric cancer. In contrast to their disappointing results *in vitro*[46], they observed enhanced anti-tumor effects by HSV-tk/GCV suicide gene therapy combined with recombinant TNF-α expression *in vivo*[44]. Using a similar protocol, another group found strongly enhanced anti-tumor effects after coexpression of IL-2, GM-CSF and HSV-tk/GCV in a gastric cancer model *in vivo*[47]. These results strongly indicate the potential impact of combined cytotoxic and immunomodulatory gene therapy in gastric cancer. Other immunotherapeutics also demonstrated their potential efficacy in gastric cancer. For example, it was shown that the expression of recombinant intercellular adhesion molecule (ICAM)-2 prolonged the survival of mice with peritoneal metastases of gastric cancer[48]. Meng *et al*[49] tested the recently discovered gastric carcinoma-specific tumor-associated antigen MG7-Ag in a *Salomonella typhimurium* vaccine against gastric cancer. In detail, they constructed a recombinant gene vaccine consisting of the MG7-Ag mimotope fused with HBcAg, a protein from HBV enhancing the immunogenicity of its antigens. Oral application of the vaccine *in vivo* led to increased formation of MG7-Ag antibodies, reduced average tumor weight compared to the controls and prevented tumor growth in one of five immunized mice, thereby indicating some protective effects of the vaccine[50].

GENE SILENCING APPROACHES

Inappropriately expressed genes are a major cause of uncontrolled cell growth. Thus, the specific downregulation of (onco)gene expression leading to tumor growth inhibition is a promising approach in cancer gene therapy[51]. Several years ago, double-strand RNA molecules homologous to the sequence of the target gene were shown to induce post-transcriptional gene silencing (PTGS) in a sequence-specific manner. This mechanism was designated as RNAi[52]. The process of PTGS is initiated by small interfering (si) RNA molecules, which have a length of 21-23 nucleotides[12]. In mammalian cells, siRNAs are incorporated into a large protein complex, the RNA-induced silencing complex, leading to precise degradation of complementary mRNA targets[53]. Due to its extraordinary efficiency, target gene specificity and simplicity of construction, siRNA technology has gained considerable attention as a new tool for gene knockdown and, hence, therapeutic use in cancer gene therapy. Chemically synthesized or *in vitro*-transcribed siRNAs are widely used for *in vitro* anti-cancer studies, while their use *in vivo* revealed several problems. Major limitations *in vivo* are the generally low transduction efficiency and short half-life. Furthermore, synthetic siRNAs preferentially transduce the liver after systemic application[54], rendering them useless for systemic cancer gene therapy. These obstacles may be overcome by the expression of siRNA from viral vectors. Currently, adenoviral, retroviral and adeno-associated virus vectors have been shown to efficiently express siRNAs resulting in strong downregulation of the target gene[55-57]. In this setting, vector-based expression systems were further developed, enabling tissue-specific and inducible siRNA expression by the use of tissue specific promoters[58] and pharmacologically regulated gene expression systems[55].

Furthermore, siRNA expressed from viral vectors seems to be more stable than synthetic siRNA[59]. Several studies were undertaken to evaluate siRNA technology in gastric cancer. Hong *et al*[60] constructed a eukaryotic vector expressing siRNA against new zinc ribbon (ZNRD1) gene, which promotes a multi-drug resistant phenotype in gastric cancer through the upregulation of permeability-glycoprotein. After transfection of a ZNRD1 siRNA, a dramatic reduction of ZNRD1 was observed accompanied by a significantly enhanced sensitivity to vincristine, adriamycin and etoposide. Further studies proved the high efficiency of siRNA-mediated gene silencing in gastric cancer cells *in vitro*[35,59,61-63]. Continuous development of siRNA technology warrants further investigations of its future therapeutic use in gastric cancer *in vivo*.

Further approaches for the downregulation of tumor genes in gastric cancer, including anti-sense-RNA[64], anti-sense oligonucleotides[65,66], ribozymes[67], and dominant negative forms of tumor proteins[37,68], have also been investigated and may be of potential clinical value in the gene therapy of gastric cancer. While anti-sense strategies preferentially aim at blocking the translation of a target mRNA by complementary binding to its specific mRNA, dominant negative mutant alleles compete with their endogenous homologs for binding in a protein complex, leading to the inhibition of protein function. For example, the insulin-like growth factor (IGF) I receptor is involved in carcinogenesis and proliferation. Its blockade by adenovector-mediated expression of a truncated dominant negative IGF was shown to sensitize gastric tumor cells for chemotherapy and to suppress their peritoneal dissemination *in vivo*[68]. In another study, Kim *et al*[66] showed that downregulation of anti-apoptotic protein bcl-2 by administering bcl-2 anti-sense oligonucleotides significantly increased the sensitivity of gastric cancer to chemotherapeutics *in vivo*.

VIROTHERAPY

The limited efficiency of replication-deficient viral vectors to transduce cancer cells and express effector genes *in vivo* led to the development of a new vector generation called OVV. In contrast to replication-deficient viral vectors, the primary replication cycle of OVV causes viro-oncolyis of initially infected tumor cells, resulting in the release of progeny virions followed by the infection of adjacent cells and the infection and destruction of further tumor mass[69]. Thus, OVV are intended to ultimately destroy a tumor although only a small percentage of tumor cells was initially infected. Furthermore, progeny virions can spread systemically by circulation[70] and infect tumor cells remote from the primary replication site of OVV, thus enhancing the potential therapeutic efficacy in metastatic cancer.

The restriction of OVV replication to cancer cells is a central concern of OVV development. This aim has been achieved by genetic engineering of viral vector genomes (e.g. in herpes- and adenoviruses) either by driving of viral genes essential for virus replication by tumor-specific promoters[71,72] or by inserting mutations into viral genes that abolish their function for viral replication in normal cells but not in tumor cells[73]. Other OVV with inherent oncolytic potency acquire tumor-selective replication competence through defects or dysregulation of cellular genes in cancer cells (e.g. Newcastle virus and vesicular stomatitis virus)[74,75].

Several studies have demonstrated that replication of OVV is 100- to 1 000-fold attenuated in normal cells compared to cancer cells. As shown in oAdVs, OVV safety can be further increased by pharmacological regulation of viral replication, using the rapamycin[76] or the Tet-On gene expression system[77,78] to regulate adenoviral E1A. This now opens the door to permanent external control of OVV during the treatment of patients. Various genetically engineered OVV and viruses with inherent oncolytic properties have recently been explored as anti-cancer agents, among them adenovirus[9,79,80], HSV[81-83], retroviruses[84], vaccinia virus[41], autonomous rodent parvovirus[85], vesicular stomatitis virus[86], Newcastle virus[87], and reoviruses[88-89]. Of these, HSV and adenovirus are the most widely studied ones. ONYX-015 was the first tested oAdV and is to date the most commonly used oAdV in clinical trials. Deletion of the adenoviral E1B-55kD enables the replication of ONYX-015 in cells with a defective p53 pathway and minimizes its replication in cells with a functionally active p53 pathway[9]. Thus, ONYX-015 is unable to replicate in normal cells, but strongly replicates in cancer cells. Several clinical trials have demonstrated the efficacy of ONYX-015 in patients with cancer. Strongest anti-tumor responses were observed in patients with squamous cell cancer of the head and neck[90,91], but responses to hepatocellular carcinoma[92], hepatobiliary tumors[93], and advanced pancreatic cancer[94] were reported, whereas no response was observed in patients with advanced ovarian cancer[95]. Two phase I/II clinical trials have provided evidence for the efficacy of ONYX-015 in metastatic gastrointestinal cancer[96,97]. Reid *et al*[96] administered ONYX-015 by hepatic artery infusion combined with 5-fluorouracil and leucoverin in 27 patients with both primary gastrointestinal carcinoma and liver metastases. The treatment was well tolerated showing only mild or moderate flu-like symptoms, including fever, myalgia, astenia and/or chills. Virus replication was demonstrated and three partial responses, four minor responses and nine stable diseases were documented as therapeutic outcome. In another study, patients with advanced sarcomas, among them patients with gastrointestinal stromal tumors, were given an intratumoral injection of ONYX-015 combined with MAP chemotherapy. The treatment was well tolerated and there was no significant toxicity. One of the six patients treated showed a partial response with an approximately 70% reduction of tumor size, and in four patients the disease stabilized[97]. Other oncolytic viruses like HSV, Newcastle virus and vaccinia virus also demonstrated their viro-oncolytic efficacy in clinical trials with cancer patients[41,98,99]. On the other hand, the studies revealed therapeutic limitations of the currently available OVV. Often, only a minority of patients shows a response, which is only partial and transient in most of the cases[5,97]. Obviously, there are several major limitations to the therapeutic potential of OVV. The key problems are low infectivity, replication rate and cytolytic activity of OVV.

To overcome these limitations, measures have therefore been taken to further develop OVV. The low transduction efficiency of oAdV due to low coxsackie-adenovirus receptor (CAR) expression can be enhanced by modifying the fiber proteins. This can be achieved by adding foreign peptides to the HI loop or the C-terminus of the fiber knob[100,101] or by substituting fibers of adenoviral 2 and 5 with fibers derived from other adenoviruses, which bind to receptor molecules other than CAR[102,103]. These strategies seem to be promising for the treatment of gastric cancer as well, since gastric cancer cells express low amounts of CAR, making it resistant to adenoviral infection[100]. Recently published data demonstrate that oAdV with RGD motif in the HI-loop of the fiber-knob region or replacing its adenovirus type 5 knob by an adenovirus type 3 knob has a stronger anti-tumor effect than unmodified oAdV in a gastric cancer model *in vivo*[100]. Another study investigated re-targeting a doubly-ablated adenovector to the epithelial cell adhesion molecule (EpCAM) by introducing a bi-specific single-chain antibody to EpCAM. EpCAM is highly expressed in gastric cancer but not in gastric epithelium. Consequently, the vector was highly selective for primary gastric tumors, while transduction of normal gastric epithelium and liver was low[104].

Another way to improve the efficacy of OVV is combining OVV treatment with conventional and other gene therapeutic strategies. Preclinical and clinical data demonstrate that OVV-induced tumor cell killing can be strongly enhanced by the expression of therapeutic transgenes from OVV like anti-angiogenetic factors, suicide genes, or tumor suppressor genes and simultaneous treatment with conventional chemo- and radiotherapy[43,96,105-110].

PROSPECTS

Gene therapy has become a generally accepted new therapeutic tool in the treatment of cancer. More and more cancer patients profit from its use due to the progress made in the development of vector systems and gene therapeutic strategies. Thus, cancer gene therapy will increase its importance as a therapeutic tool even though many problems still need to be solved. One of the most important issues affecting the possible clinical application of gene therapy is the need to ensure the highest possible safety levels. Many clinical investigations have demonstrated that the currently available vector systems are well tolerated and side effects are acceptable. However, the use of retroviral vectors is discussed controversially, since 3 of 11 children with X-linked severe combined immunodeficiency, who were treated with a retrovirus, developed uncontrolled T-lymphocyte proliferation in a French gene therapy trial.

The major problem of cancer gene therapy that still remains is the relatively poor therapeutic outcome. This problem is not restricted to a specific tumor entity, but is rather a general problem. There may be many reasons for this, but it is widely agreed that this is mainly due to the relative resistance of cancer cells to introduce foreign material combined with low transgene expression *in vivo*. Thus, improved vector systems and application protocols will continue to be the biggest issues to be dealt with in cancer gene therapy in the next few years. However, important progress to overcome these limitations has already been made by the development of OVVs and vectors with increased tumor cell tropism.

Great progress has also been made in the development of gene therapeutic strategies in gastric cancer. New vector systems as well as the evaluation of new target genes and gene therapeutic strategies have substantially improved the chances for successful treatment of gastric cancer by gene therapy. The next challenge will be to test the results gained thus far in clinical studies.

REFERENCES

1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108
2 **Murray CJ**, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* 1997; **349**: 1498-1504
3 **Gottesman MM**. Cancer gene therapy: an awkward adolescence. *Cancer Gene Ther* 2003; **10**: 501-508
4 **Heo DS**. Progress and limitations in cancer gene therapy. *Genet Med* 2002; **4**: 52S-55S
5 **Lin E**, Nemunaitis J. Oncolytic viral therapies. *Cancer Gene Ther* 2004; **11**: 643-664
6 **Fechner H**, Haack A, Wang H, Wang X, Eizema K, Pauschinger M, Schoemaker R, Veghel R, Houtsmuller A, Schultheiss HP, Lamers J, Poller W. Expression of coxsackie adenovirus receptor and alphav-integrin does not correlate with adenovector targeting in vivo indicating anatomical vector barriers. *Gene Ther* 1999; **6**: 1520-1535
7 **Okegawa T**, Li Y, Pong RC, Bergelson JM, Zhou J, Hsieh JT. The dual impact of coxsackie and adenovirus receptor expression on human prostate cancer gene therapy. *Cancer Res* 2000; **60**: 5031-5036
8 **Alemany R**, Suzuki K, Curiel DT. Blood clearance rates of adenovirus type 5 in mice. *J Gen Virol* 2000; **81**: 2605-2609
9 **Bischoff JR**, Kirn DH, Williams A, Heise C, Horn S, Muna M, Ng L, Nye JA, Sampson-Johannes A, Fattaey A, McCormick F. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* 1996; **274**: 373-376
10 **Mizuguchi H**, Hayakawa T. Targeted adenovirus vectors. *Hum Gene Ther* 2004; **15**: 1034-1044
11 **Grandi P**, Spear M, Breakefield XO, Wang S. Targeting HSV amplicon vectors. *Methods* 2004; **33**: 179-186
12 **Elbashir SM**, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature* 2001; **411**: 494-498
13 **Yen N**, Ioannides CG, Xu K, Swisher SG, Lawrence DD, Kemp BL, El-Naggar AK, Cristiano RJ, Fang B, Glisson BS, Hong WK, Khuri FR, Kurie JM, Lee JJ, Lee JS, Merritt JA, Mukhopadhyay T, Nesbitt JC, Nguyen D, Perez-Soler R, Pisters KM, Putnam JB, Schrump DS, Shin DM, Walsh GL, Roth JA. Cellular and humoral immune responses to adenovirus and p53 protein antigens in patients following intratumoral injection of an adenovirus vector expressing wild-type. P53 (Ad-p53). *Cancer Gene Ther* 2000; **7**: 530-536
14 **Horio Y**, Hasegawa Y, Sekido Y, Takahashi M, Roth JA, Shimokata K. Synergistic effects of adenovirus expressing wild-type p53 on chemosensitivity of non-small cell lung cancer cells. *Cancer Gene Ther* 2000; **7**: 537-544
15 **Tatebe S**, Matsuura T, Endo K, Teramachi K, Nakamura T, Sato K, Ito H. Adenovirus-mediated transfer of wild-type p53 gene results in apoptosis or growth arrest in human cultured gastric carcinoma cells. *Int J Oncol* 1999; **15**: 229-235
16 **Ohashi M**, Kanai F, Ueno H, Tanaka T, Tateishi K, Kawakami T, Koike Y, Ikenoue T, Shiratori Y, Hamada H, Omata M. Adenovirus mediated p53 tumour suppressor gene therapy

for human gastric cancer cells in vitro and in vivo. *Gut* 1999; **44**: 366-371
17 **Wolf BB**, Schuler M, Li W, Eggers-Sedlet B, Lee W, Tailor P, Fitzgerald P, Mills GB, Green DR. Defective cytochrome c-dependent caspase activation in ovarian cancer cell lines due to diminished or absent apoptotic protease activating factor-1 activity. *J Biol Chem* 2001; **276**: 34244-34251
18 **Tsunemitsu Y**, Kagawa S, Tokunaga N, Otani S, Umeoka T, Roth JA, Fang B, Tanaka N, Fujiwara T. Molecular therapy for peritoneal dissemination of xenotransplanted human MKN-45 gastric cancer cells with adenovirus mediated Bax gene transfer. *Gut* 2004; **53**: 554-560
19 **Nishimura S**, Adachi M, Ishida T, Matsunaga T, Uchida H, Hamada H, Imai K. Adenovirus-mediated transfection of caspase-8 augments anoikis and inhibits peritoneal dissemination of human gastric carcinoma cells. *Cancer Res* 2001; **61**: 7009-7014
20 **Fu YG**, Qu YJ, Wu KC, Zhai HH, Liu ZG, Fan DM. Apoptosis-inducing effect of recombinant Caspase-3 expressed by constructed eukaryotic vector on gastric cancer cell line SGC7901. *World J Gastroenterol* 2003; **9**: 1935-1939
21 **Jeong YW**, Kim KS, Oh JY, Park JC, Baek WK, Suh SI, Suh MH, Lee JC, Cho JW. Exogenous wild-type p16INK4A gene induces delayed cell proliferation and promotes chemosensitivity through decreased pRB and increased E2F-1 expressions. *Int J Mol Med* 2003; **12**: 61-65
22 **Ishii H**, Zanesi N, Vecchione A, Trapasso F, Yendamuri S, Sarti M, Baffa R, During MJ, Huebner K, Fong LY, Croce CM. Regression of upper gastric cancer in mice by FHIT gene delivery. *FASEB J* 2003; **17**: 1768-1770
23 **Kirn D**, Niculescu-Duvaz I, Hallden G, Springer CJ. The emerging fields of suicide gene therapy and virotherapy. *Trends Mol Med* 2002; **8**: S68-S73
24 **Wei SJ**, Chao Y, Hung YM, Lin WC, Yang DM, Shih YL, Ch'ang LY, Whang-Peng J, Yang WK. S- and G2-phase cell cycle arrests and apoptosis induced by ganciclovir in murine melanoma cells transduced with herpes simplex virus thymidine kinase. *Exp Cell Res* 1998; **241**: 66-75
25 **Kwon GY**, Jeong J, Woo JK, Choi HY, Lee MJ, Ko JK, Shim YH, Kim CW. Co-expression of bfl-1 enhances host response in the herpes simplex virus-thymidine kinase/ganciclovir gene therapy system. *Biochem Biophys Res Commun* 2003; **303**: 756-763
26 **Freeman SM**, Abboud CN, Whartenby KA, Packman CH, Koeplin DS, Moolten FL, Abraham GN. The "bystander effect": tumor regression when a fraction of the tumor mass is genetically modified. *Cancer Res* 1993; **53**: 5274-5283
27 **Tanaka T**, Yamasaki H, Mesnil M. Induction of a bystander effect in HeLa cells by using a bigenic vector carrying viral thymidine kinase and connexin32 genes. *Mol Carcinog* 2001; **30**: 176-180
28 **Nakaya H**, Ishizu A, Ikeda H, Tahara M, Shindo J, Itoh R, Takahashi T, Asaka M, Ishikura H, Yoshiki T. In vitro model of suicide gene therapy for alpha-fetoprotein-producing gastric cancer. *Anticancer Res* ; **23**: 3795-3800
29 **Aberle S**, Schug N, Mathlouthi R, Seitz G, Küpper JH, Schröder K, Blin N. Promoter selection for the cytosine deaminase suicide gene constructs in gastric cancer. *Eur J Gastroenterol Hepatol* 2004; **16**: 63-67
30 **Ueda K**, Iwahashi M, Nakamori M, Nakamura M, Matsuura I, Yamaue H, Tanimura H. Carcinoembryonic antigen-specific suicide gene therapy of cytosine deaminase/5-fluorocytosine enhanced by the cre/loxP system in the orthotopic gastric carcinoma model. *Cancer Res* 2001; **61**: 6158-6162
31 **Chung-Faye G**, Palmer D, Anderson D, Clark J, Downes M, Baddeley J, Hussain S, Murray PI, Searle P, Seymour L, Harris PA, Ferry D, Kerr DJ. Virus-directed, enzyme prodrug therapy with nitroimidazole reductase: a phase I and pharmacokinetic study of its prodrug, CB1954. *Clin Cancer Res* 2001; **7**: 2662-2668
32 **Tandle A**, Blazer DG, Libutti SK. Antiangiogenic gene therapy of cancer: recent developments. *J Transl Med* 2004; **2**: 22
33 **Sako A**, Kitayama J, Koyama H, Ueno H, Uchida H, Hamada H, Nagawa H. Transduction of soluble Flt-1 gene to peritoneal mesothelial cells can effectively suppress peritoneal metastasis of gastric cancer. *Cancer Res* 2004; **64**: 3624-3628
34 **Ueda K**, Iwahashi M, Matsuura I, Nakamori M, Nakamura M, Ojima T, Naka T, Ishida K, Matsumoto K, Nakamura T, Yamaue H. Adenoviral-mediated gene transduction of the hepatocyte growth factor (HGF) antagonist, NK4, suppresses peritoneal metastases of gastric cancer in nude mice. *Eur J Cancer* 2004; **40**: 2135-2142
35 **Meng F**, Ding J, Liu N, Zhang J, Shao X, Shen H, Xue Y, Xie H, Fan D. Inhibition of gastric cancer angiogenesis by vector-based RNA interference for Raf-1. *Cancer Biol Ther* 2005; **4**: 113-117
36 **Xue Y**, Bi F, Zhang X, Pan Y, Liu N, Zheng Y, Fan D. Inhibition of endothelial cell proliferation by targeting Rac1 GTPase with small interference RNA in tumor cells. *Biochem Biophys Res Commun* 2004; **320**: 1309-1315
37 **Stoeltzing O**, McCarty MF, Wey JS, Fan F, Liu W, Belcheva A, Bucana CD, Semenza GL, Ellis LM. Role of hypoxia-inducible factor 1alpha in gastric cancer cell growth, angiogenesis, and vessel maturation. *J Natl Cancer Inst* 2004; **96**: 946-956
38 **Larin SS**, Georgiev GP, Kiselev SL. Gene transfer approaches in cancer immunotherapy. *Gene Ther* 2004; **11 Suppl 1**: S18-S25
39 **Narvaiza I**, Mazzolini G, Barajas M, Duarte M, Zaratiegui M, Qian C, Melero I, Prieto J. Intratumoral coinjection of two adenoviruses, one encoding the chemokine IFN-gamma-inducible protein-10 and another encoding IL-12, results in marked antitumoral synergy. *J Immunol* 2000; **164**: 3112-3122
40 **Kircheis R**, Küpcü Z, Wallner G, Wagner E. Cytokine gene-modified tumor cells for prophylactic and therapeutic vaccination: IL-2, IFN-gamma, or combination IL-2 + IFN-gamma. *Cytokines Cell Mol Ther* 1998; **4**: 95-103
41 **Mastrangelo MJ**, Maguire HC, Eisenlohr LC, Laughlin CE, Monken CE, McCue PA, Kovatich AJ, Lattime EC. Intratumoral recombinant GM-CSF-encoding virus as gene therapy in patients with cutaneous melanoma. *Cancer Gene Ther* ; **6**: 409-422
42 **Liu Y**, Saxena A, Zheng C, Carlsen S, Xiang J. Combined alpha tumor necrosis factor gene therapy and engineered dendritic cell vaccine in combating well-established tumors. *J Gene Med* 2004; **6**: 857-868
43 **Motoi F**, Sunamura M, Ding L, Duda DG, Yoshida Y, Zhang W, Matsuno S, Hamada H. Effective gene therapy for pancreatic cancer by cytokines mediated by restricted replication-competent adenovirus. *Hum Gene Ther* 2000; **11**: 223-235
44 **Zhang JH**, Wan MX, Pan BR, Yu B. Cytotoxicity of HSVtk and hrTNF-alpha fusion genes with IRES in treatment of gastric cancer. *Cancer Biol Ther* 2004; **3**: 1075-1080
45 **Zhang JH**, Wan MX, Yuan JY, Pan BR. Construction and identification of recombinant vectors carrying herpes simplex virus thymidine kinase and cytokine genes expressed in gastric carcinoma cell line SGC7901. *World J Gastroenterol* 2004; **10**: 26-30
46 **Zhang JH**, Wan MX, Yuan JY, Pan BR. Do there exist synergistic antitumor effects by coexpression of herpes simplex virus thymidine kinase with cytokine genes on human gastric cancer cell line SGC7901? *World J Gastroenterol* 2004; **10**: 147-151
47 **Guo SY**, Gu QL, Zhu ZG, Hong HQ, Lin YZ. TK gene combined with mIL-2 and mGM-CSF genes in treatment of gastric cancer. *World J Gastroenterol* 2003; **9**: 233-237
48 **Tanaka H**, Yashiro M, Sunami T, Sakate Y, Kosaka K, Hirakawa K. ICAM-2 gene therapy for peritoneal dissemination of scirrhous gastric carcinoma. *Clin Cancer Res* 2004; **10**: 4885-4892
49 **Meng FP**, Ding J, Yu ZC, Han QL, Guo CC, Liu N, Fan DM. Oral attenuated Salmonella typhimurium vaccine against MG7-Ag mimotope of gastric cancer. *World J Gastroenterol* 2005; **11**: 1833-1836
50 **Schödel F**, Moriarty AM, Peterson DL, Zheng JA, Hughes JL, Will H, Leturcq DJ, McGee JS, Milich DR. The position of heterologous epitopes inserted in hepatitis B virus core particles determines their immunogenicity. *J Virol* 1992; **66**: 106-114
51 **Ryther RC**, Flynt AS, Phillips JA, Patton JG. siRNA therapeutics: big potential from small RNAs. *Gene Ther* 2005; **12**: 5-11
52 **Fire A**, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC. Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* 1998; **391**: 806-811
53 **Tuschl T**. Expanding small RNA interference. *Nat Biotechnol*

2002; **20**: 446-448
54 **Lewis DL**, Hagstrom JE, Loomis AG, Wolff JA, Herweijer H. Efficient delivery of siRNA for inhibition of gene expression in postnatal mice. *Nat Genet* 2002; **32**: 107-108
55 **Hosono T**, Mizuguchi H, Katayama K, Xu ZL, Sakurai F, Ishii-Watabe A, Kawabata K, Yamaguchi T, Nakagawa S, Mayumi T, Hayakawa T. Adenovirus vector-mediated doxycycline-inducible RNA interference. *Hum Gene Ther* 2004; **15**: 813-819
56 **Sumimoto H**, Yamagata S, Shimizu A, Miyoshi H, Mizuguchi H, Hayakawa T, Miyagishi M, Taira K, Kawakami Y. Gene therapy for human small-cell lung carcinoma by inactivation of Skp-2 with virally mediated RNA interference. *Gene Ther* 2005; **12**: 95-100
57 **Xu D**, McCarty D, Fernandes A, Fisher M, Samulski RJ, Juliano RL. Delivery of MDR1 small interfering RNA by self-complementary recombinant adeno-associated virus vector. *Mol Ther* 2005; **11**: 523-530
58 **Song J**, Pang S, Lu Y, Yokoyama KK, Zheng JY, Chiu R. Gene silencing in androgen-responsive prostate cancer cells from the tissue-specific prostate-specific antigen promoter. *Cancer Res* 2004; **64**: 7661-7663
59 **Shinomiya N**, Gao CF, Xie Q, Gustafson M, Waters DJ, Zhang YW, Vande Woude GF. RNA interference reveals that ligand-independent met activity is required for tumor cell signaling and survival. *Cancer Res* 2004; **64**: 7962-7970
60 **Hong L**, Ning X, Shi Y, Shen H, Zhang Y, Lan M, Liang S, Wang J, Fan D. Reversal of multidrug resistance of gastric cancer cells by down-regulation of ZNRD1 with ZNRD1 siRNA. *Br J Biomed Sci* 2004; **61**: 206-210
61 **Liu N**, Bi F, Pan Y, Sun L, Xue Y, Shi Y, Yao X, Zheng Y, Fan D. Reversal of the malignant phenotype of gastric cancer cells by inhibition of RhoA expression and activity. *Clin Cancer Res* 2004; **10**: 6239-6247
62 **Nieth C**, Priebsch A, Stege A, Lage H. Modulation of the classical multidrug resistance (MDR) phenotype by RNA interference (RNAi). *FEBS Lett* 2003; **545**: 144-150
63 **Guo C**, Ding J, Yao L, Sun L, Lin T, Song Y, Sun L, Fan D. Tumor suppressor gene Runx3 sensitizes gastric cancer cells to chemotherapeutic drugs by downregulating Bcl-2, MDR-1 and MRP-1. *Int J Cancer* 2005; **116**: 155-160
64 **Tong QS**, Zheng LD, Wang L, Zeng FQ, Chen FM, Dong JH, Lu GC. Downregulation of XIAP expression induces apoptosis and enhances chemotherapeutic sensitivity in human gastric cancer cells. *Cancer Gene Ther* 2005; **12**: 509-514
65 **Ye J**, Wu YL, Zhang S, Chen Z, Guo LX, Zhou RY, Xie H. Inhibitory effect of human telomerase antisense oligodeoxy ribonucleotides on the growth of gastric cancer cell lines in variant tumor pathological subtype. *World J Gastroenterol* 2005; **11**: 2230-2237
66 **Kim R**, Emi M, Tanabe K, Toge T. Preclinical evaluation of antisense bcl-2 as a chemosensitizer for patients with gastric carcinoma. *Cancer* 2004; **101**: 2177-2186
67 **Bi F**, Fan D, Hui H, Wang C, Zhang X. Reversion of the malignant phenotype of gastric cancer cell SGC7901 by c-erbB-2-specific hammerhead ribozyme. *Cancer Gene Ther* 2001; **8**: 835-842
68 **Min Y**, Adachi Y, Yamamoto H, Imsumran A, Arimura Y, Endo T, Hinoda Y, Lee CT, Nadaf S, Carbone DP, Imai K. Insulin-like growth factor I receptor blockade enhances chemotherapy and radiation responses and inhibits tumour growth in human gastric cancer xenografts. *Gut* 2005; **54**: 591-600
69 **Bauerschmitz GJ**, Barker SD, Hemminki A. Adenoviral gene therapy for cancer: from vectors to targeted and replication competent agents (review). *Int J Oncol* 2002; **21**: 1161-1174
70 **Sauthoff H**, Hu J, Maca C, Goldman M, Heitner S, Yee H, Pipiya T, Rom WN, Hay JG. Intratumoral spread of wild-type adenovirus is limited after local injection of human xenograft tumors: virus persists and spreads systemically at late time points. *Hum Gene Ther* 2003; **14**: 425-433
71 **Nettelbeck DM**, Rivera AA, Balagué C, Alemany R, Curiel DT. Novel oncolytic adenoviruses targeted to melanoma: specific viral replication and cytolysis by expression of E1A mutants from the tyrosinase enhancer/promoter. *Cancer Res* 2002; **62**: 4663-4670
72 **Huang TG**, Savontaus MJ, Shinozaki K, Sauter BV, Woo SL. Telomerase-dependent oncolytic adenovirus for cancer treatment. *Gene Ther* 2003; **10**: 1241-1247
73 **Heise C**, Hermiston T, Johnson L, Brooks G, Sampson-Johannes A, Williams A, Hawkins L, Kirn D. An adenovirus E1A mutant that demonstrates potent and selective systemic anti-tumoral efficacy. *Nat Med* 2000; **6**: 1134-1139
74 **Stojdl DF**, Lichty B, Knowles S, Marius R, Atkins H, Sonenberg N, Bell JC. Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. *Nat Med* 2000; **6**: 821-825
75 **McKie EA**, MacLean AR, Lewis AD, Cruickshank G, Rampling R, Barnett SC, Kennedy PG, Brown SM. Selective in vitro replication of herpes simplex virus type 1 (HSV-1) ICP34.5 null mutants in primary human CNS tumours--evaluation of a potentially effective clinical therapy. *Br J Cancer* 1996; **74**: 745-752
76 **Chong H**, Ruchatz A, Clackson T, Rivera VM, Vile RG. A system for small-molecule control of conditionally replication-competent adenoviral vectors. *Mol Ther* 2002; **5**: 195-203
77 **Fechner H**, Wang X, Srour M, Siemetzki U, Seltmann H, Sutter AP, Scherübl H, Zouboulis CC, Schwaab R, Hillen W, Schultheiss HP, Poller W. A novel tetracycline-controlled transactivator-transrepressor system enables external control of oncolytic adenovirus replication. *Gene Ther* 2003; **10**: 1680-1690
78 **Hurtado Picó A**, Wang X, Sipo I, Siemetzki U, Eberle J, Poller W, Fechner H. Viral and nonviral factors causing nonspecific replication of tumor- and tissue-specific promoter-dependent oncolytic adenoviruses. *Mol Ther* 2005; **11**: 563-577
79 **Rodriguez R**, Schuur ER, Lim HY, Henderson GA, Simons JW, Henderson DR. Prostate attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate-specific antigen-positive prostate cancer cells. *Cancer Res* 1997; **57**: 2559-2563
80 **Toth K**, Djeha H, Ying B, Tollefson AE, Kuppuswamy M, Doronin K, Krajcsi P, Lipinski K, Wrighton CJ, Wold WS. An oncolytic adenovirus vector combining enhanced cell-to-cell spreading, mediated by the ADP cytolytic protein, with selective replication in cancer cells with deregulated wnt signaling. *Cancer Res* 2004; **64**: 3638-3644
81 **Kambara H**, Okano H, Chiocca EA, Saeki Y. An oncolytic HSV-1 mutant expressing ICP34.5 under control of a nestin promoter increases survival of animals even when symptomatic from a brain tumor. *Cancer Res* 2005; **65**: 2832-2839
82 **Reinblatt M**, Pin RH, Fong Y. Carcinoembryonic antigen directed herpes viral oncolysis improves selectivity and activity in colorectal cancer. *Surgery* 2004; **136**: 579-584
83 **Walker JR**, McGeagh KG, Sundaresan P, Jorgensen TJ, Rabkin SD, Martuza RL. Local and systemic therapy of human prostate adenocarcinoma with the conditionally replicating herpes simplex virus vector G207. *Hum Gene Ther* 1999; **10**: 2237-2243
84 **Wang WJ**, Tai CK, Kasahara N, Chen TC. Highly efficient and tumor-restricted gene transfer to malignant gliomas by replication-competent retroviral vectors. *Hum Gene Ther* 2003; **14**: 117-127
85 **Olijslagers S**, Dege AY, Dinsart C, Voorhoeve M, Rommelaere J, Noteborn MH, Cornelis JJ. Potentiation of a recombinant oncolytic parvovirus by expression of Apoptin. *Cancer Gene Ther* 2001; **8**: 958-965
86 **Shinozaki K**, Ebert O, Woo SL. Eradication of advanced hepatocellular carcinoma in rats via repeated hepatic arterial infusions of recombinant VSV. *Hepatology* 2005; **41**: 196-203
87 **Phuangsab A**, Lorence RM, Reichard KW, Peeples ME, Walter RJ. Newcastle disease virus therapy of human tumor xenografts: antitumor effects of local or systemic administration. *Cancer Lett* 2001; **172**: 27-36
88 **Coffey MC**, Strong JE, Forsyth PA, Lee PW. Reovirus therapy of tumors with activated Ras pathway. *Science* 1998; **282**:

1332-1334
89 **Norman KL**, Coffey MC, Hirasawa K, Demetrick DJ, Nishikawa SG, DiFrancesco LM, Strong JE, Lee PW. Reovirus oncolysis of human breast cancer. *Hum Gene Ther* 2002; **13**: 641-652
90 **Khuri FR**, Nemunaitis J, Ganly I, Arseneau J, Tannock IF, Romel L, Gore M, Ironside J, MacDougall RH, Heise C, Randlev B, Gillenwater AM, Bruso P, Kaye SB, Hong WK, Kirn DH. a controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat Med* 2000; **6**: 879-885
91 **Nemunaitis J**, Khuri F, Ganly I, Arseneau J, Posner M, Vokes E, Kuhn J, McCarty T, Landers S, Blackburn A, Romel L, Randlev B, Kaye S, Kirn D. Phase II trial of intratumoral administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer. *J Clin Oncol* 2001; **19**: 289-298
92 **Habib NA**, Mitry RR, Sarraf CE, Jiao LR, Havlík R, Nicholls J, Jensen SL. Assessment of growth inhibition and morphological changes in in vitro and in vivo hepatocellular carcinoma models post treatment with dl1520 adenovirus. *Cancer Gene Ther* 2002; **9**: 414-420
93 **Makower D**, Rozenblit A, Kaufman H, Edelman M, Lane ME, Zwiebel J, Haynes H, Wadler S. Phase II clinical trial of intralesional administration of the oncolytic adenovirus ONYX-015 in patients with hepatobiliary tumors with correlative p53 studies. *Clin Cancer Res* 2003; **9**: 693-702
94 **Hecht JR**, Bedford R, Abbruzzese JL, Lahoti S, Reid TR, Soetikno RM, Kirn DH, Freeman SM. A phase I/II trial of intratumoral endoscopic ultrasound injection of ONYX-015 with intravenous gemcitabine in unresectable pancreatic carcinoma. *Clin Cancer Res* 2003; **9**: 555-561
95 **Vasey PA**, Shulman LN, Campos S, Davis J, Gore M, Johnston S, Kirn DH, O'Neill V, Siddiqui N, Seiden MV, Kaye SB. Phase I trial of intraperitoneal injection of the E1B-55-kd-gene-deleted adenovirus ONYX-015 (dl1520) given on days 1 through 5 every 3 weeks in patients with recurrent/refractory epithelial ovarian cancer. *J Clin Oncol* 2002; **20**: 1562-1569
96 **Reid T**, Galanis E, Abbruzzese J, Sze D, Wein LM, Andrews J, Randlev B, Heise C, Uprichard M, Hatfield M, Rome L, Rubin J, Kirn D. Hepatic arterial infusion of a replication-selective oncolytic adenovirus (dl1520): phase II viral, immunologic, and clinical endpoints. *Cancer Res* 2002; **62**: 6070-6079
97 **Galanis E**, Okuno SH, Nascimento AG, Lewis BD, Lee RA, Oliveira AM, Sloan JA, Atherton P, Edmonson JH, Erlichman C, Randlev B, Wang Q, Freeman S, Rubin J. Phase I-II trial of ONYX-015 in combination with MAP chemotherapy in patients with advanced sarcomas. *Gene Ther* 2005; **12**: 437-445
98 **Bennett JJ**, Delman KA, Burt BM, Mariotti A, Malhotra S, Zager J, Petrowsky H, Mastorides S, Federoff H, Fong Y. Comparison of safety, delivery, and efficacy of two oncolytic herpes viruses (G207 and NV1020) for peritoneal cancer. *Cancer Gene Ther* 2002; **9**: 935-945
99 **Pecora AL**, Rizvi N, Cohen GI, Meropol NJ, Sterman D, Marshall JL, Goldberg S, Gross P, O'Neil JD, Groene WS, Roberts MS, Rabin H, Bamat MK, Lorence RM. Phase I trial of intravenous administration of PV701, an oncolytic virus, in patients with advanced solid cancers. *J Clin Oncol* 2002; **20**: 2251-2266
100 **Ono HA**, Davydova JG, Adachi Y, Takayama K, Barker SD, Reynolds PN, Krasnykh VN, Kunisaki C, Shimada H, Curiel DT, Yamamoto M. Promoter-controlled infectivity-enhanced conditionally replicative adenoviral vectors for the treatment of gastric cancer. *J Gastroenterol* 2005; **40**: 31-42
101 **Jacob D**, Bahra M, Schumacher G, Neuhaus P, Fang B. Gene therapy in colon cancer cells with a fiber-modified adenovector expressing the TRAIL gene driven by the hTERT promoter. *Anticancer Res* ; **24**: 3075-3079
102 **Mizuguchi H**, Hayakawa T. Adenovirus vectors containing chimeric type 5 and type 35 fiber proteins exhibit altered and expanded tropism and increase the size limit of foreign genes. *Gene* 2002; **285**: 69-77
103 **Denby L**, Work LM, Graham D, Hsu C, von Seggern DJ, Nicklin SA, Baker AH. Adenoviral serotype 5 vectors pseudotyped with fibers from subgroup D show modified tropism in vitro and in vivo. *Hum Gene Ther* 2004; **15**: 1054-1064
104 **Heideman DA**, van Beusechem VW, Offerhaus GJ, Wickham TJ, Roelvink PW, Craanen ME, Pinedo HM, Meijer CJ, Gerritsen WR. Selective gene transfer into primary human gastric tumors using epithelial cell adhesion molecule-targeted adenoviral vectors with ablated native tropism. *Hum Gene Ther* 2002; **13**: 1677-1685
105 **Rein DT**, Breidenbach M, Kirby TO, Han T, Siegal GP, Bauerschmitz GJ, Wang M, Nettelbeck DM, Tsuruta Y, Yamamoto M, Dall P, Hemminki A, Curiel DT. A fiber-modified, secretory leukoprotease inhibitor promoter-based conditionally replicating adenovirus for treatment of ovarian cancer. *Clin Cancer Res* 2005; **11**: 1327-1335
106 **Kim SH**, Wong RJ, Kooby DA, Carew JF, Adusumilli PS, Patel SG, Shah JP, Fong Y. Combination of mutated herpes simplex virus type 1 (G207 virus) with radiation for the treatment of squamous cell carcinoma of the head and neck. *Eur J Cancer* 2005; **41**: 313-322
107 **Lamfers ML**, Grill J, Dirven CM, Van Beusechem VW, Geoerger B, Van Den Berg J, Alemany R, Fueyo J, Curiel DT, Vassal G, Pinedo HM, Vandertop WP, Gerritsen WR. Potential of the conditionally replicative adenovirus Ad5-Delta24RGD in the treatment of malignant gliomas and its enhanced effect with radiotherapy. *Cancer Res* 2002; **62**: 5736-5742
108 **Li Y**, Yu DC, Chen Y, Amin P, Zhang H, Nguyen N, Henderson DR. A hepatocellular carcinoma-specific adenovirus variant, CV890, eliminates distant human liver tumors in combination with doxorubicin. *Cancer Res* 2001; **61**: 6428-6436
109 **Zhang Z**, Zou W, Wang J, Gu J, Dang Y, Li B, Zhao L, Qian C, Qian Q, Liu X. Suppression of tumor growth by oncolytic adenovirus-mediated delivery of an antiangiogenic gene, soluble Flt-1. *Mol Ther* 2005; **11**: 553-562
110 **Geoerger B**, Vassal G, Opolon P, Dirven CM, Morizet J, Laudani L, Grill J, Giaccone G, Vandertop WP, Gerritsen WR, van Beusechem VW. Oncolytic activity of p53-expressing conditionally replicative adenovirus AdDelta24-p53 against human malignant glioma. *Cancer Res* 2004; **64**: 5753-5759

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BASIC RESEARCH

Effect of nuclear factor kappa B on intercellular adhesion molecule-1 expression and neutrophil infiltration in lung injury induced by intestinal ischemia/reperfusion in rats

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Abstract

AIM: To investigate the role of nuclear factor kappa B (NF-κB) in the pathogenesis of lung injury induced by intestinal ischemia/reperfusion (I/R), and its effect on intercellular adhesion molecule-1 (ICAM-1) expression and neutrophil infiltration.

METHODS: Twenty-four Wistar rats were divided randomly into control, I/R and pyrrolidine dithiocarbamate (PDTC) treatment groups, $n = 8$ in each. I/R group and PDTC treatment group received superior mysenteric artery (SMA) occluding for 1 h and reperfusion for 2 h. PDTC group was administrated with intraperitoneal injection of 2% 100 mg/kg PDTC 1 h before surgery. Lung histology and bronchia alveolus lung fluid (BALF) protein were assayed. Serum IL-6, lung malondialdehyde (MDA) and myeloperoxidase (MPO) as well as the expression level of NF-κB and ICAM-1 were measured.

RESULTS: Lung injury induced by intestinal I/R, was characterized by edema, hemorrhage and neutrophil infiltration as well as by the significant rising of BALF protein. Compared to control group, the levels of serum IL-6 and lung MDA and MPO increased significantly in I/R group ($P = 0.001$). Strong positive expression of NF-κB p65 and ICAM-1 was observed. After the administration of PDTC, the level of serum IL-6, lung MDA and MPO as well as NF-κB and ICAM-1 decreased significantly ($P < 0.05$) when compared to I/R group.

CONCLUSION: The activation of NF-κB plays an important role in the pathogenesis of lung injury induced by intestinal I/R through upregulating the neutrophil infiltration and lung ICAM-1 expression. PDTC as an inhibitor of NF-κB can prevent lung injury induced by intestinal I/R through inhibiting the activity of NF-κB.

Key words: Lung injury; Intestinal ischemia/reperfusion; NF-κB; ICAM-1; Neutrophil infiltration

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INTRODUCTION

Intestinal I/R is not necessarily limited to the intestine itself, but involves severe destuction of distant tissue. It is known that intestinal I/R is an important event in the pathogenesis of multi-system organ failure syndrome, especially acute respiratory distress syndrome which is the leading cause of death in critically ill patients[1-4]. The mechanism of lung injury induced by intestinal I/R is complex. Proinflammatory cytokines and chemokines exert their effects via a direct toxic action on target cells[5], ICAM-1 and neutrophil infiltration play an important role in lung injury induced by intestinal I/R[6]. However, recent studies showed that these proinflammatory mediators play a role in gene induction[7-9]. NF-κB upregulates most of these mediators[10-12], but it is not known how NF-κB is activated and ICAM-1 is expressed in lung injury induced by intestinal ischemia.

This study was to evaluate the role of NF-κB in the pathogenesis of lung injury induced by intestinal I/R and the effect of pyrrolidine dithiocarbamate (PDTC) on lung neutrophil infiltration and expression of ICAM-1.

MATERIALS AND METHODS

Animals

Male Wistar rats (From Animal Center of Dalian Medical

University, Dalian, China) weighing 200-240 g were used in this study. All rats had free access to standard laboratory chow and water in accordance with institutional animal care policies.

Experimental design

The rats were anesthetized with intraperitoneal administration of 10% chloral hydrate, 350 mg/kg, laparotomized and randomly divided into three experimental groups ($n = 8$ in each): sham operation group (control group) undergoing full surgical preparation including isolation of SMA without occlusion; I/R group: ischemia was induced for 1 h and reperfusion for 2 h after SMA was isolated and ischemia was occluded[13]; PDTC treatment group undergoing full surgical preparation including isolation of SMA with intraperitoneal administration of 2% PDTC 100 mg/kg 1 h before the operation. The rats in control and I/R groups were treated with an equal volume of normal saline solution. All animals were killed after 2 h of reperfusion. Blood samples were obtained for analysis. Lung tissues were harvested immediately for detection.

Lung histology and bronchia alveolus lung fluid (BALF) assay

The harvested right middle lobe of the lung was fixed in 40 g/L formaldehyde. After being embedded in paraffin, 4-μm-thick sections were stained with hematoxylin and eosin for light microscopy. Pathological injury score was evaluated according to Chiu's method[14]. BALF was collected according to the process of Cox[15] and centrifuged at 1 000 r/min for 10 min. The protein in the supernatant was measured using assay kit (Nanjing Jincheng Corp., China) following the manufacturer's instructions and expressed as g/L.

Serum IL-6 assay

The serum level of IL-6 was determined using an RIA kit (Radioimmunity Institute of PLA General Hospital, Beijing, China) according to the manufacturer's instructions and expressed as ng/L.

Lung MDA and MPO assay

The right base lobe of lung was harvested and immediately homogenized on ice in 5 volumes of normal saline. The homogenates were centrifuged at 1 200 r/min for 10 min. The malondialdehyde (MDA) and myeloperoxidase (MPO) content in the supernatants were measured using MDA and MPO assay kit (Nanjing Jincheng Corp., China) following the manufacturer's instructions and expressed as nmol/mg and U/g, respectively.

Lung NF-κB and ICAM-1 immunohistochemical analysis

Formalin-fixed and paraffin-embedded lung specimens were stained with SP immunohistochemistry technique for NF-κB and ICAM-1 detection. Experiments were performed following the manufacturer's instructions. Five-micron sections were dewaxed in xylene, cultured in 3% hydrogen peroxide to eliminate intrinsic peroxidase and quenched in normal goat serum for 30 min, then incubated overnight at 4 °C with polyclonal rabbit anti-rat NF-κB p65 and ICAM-1 antibody (NeoMarkers Corp. and Boster Corp., Ltd., respectively) against purified recombinant NF-κB or ICAM-1. Then anti-rabbit immunoglobulin and streptavidin conjugated to horseradish peroxides were added. Finally, 3,3'-diaminobenzidine was used for color development and hematoxylin was used for counter staining. The results were evaluated semi-quantitatively according to the percentage of positive cells in five high power fields at 400 multiple signal magnification.

Western blot analysis of NF-κB and ICAM-1

Cellular plasma and nuclear protein were extracted from frozen lung tissue with protein extraction kit (Pierce, Meridian Road, Rockford, IL, USA) according to the manufacturer's instructions. Protein concentrations were determined by Coomassie blue dye-binding assay (Nanjing Jincheng Corp, China). Samples were mixed with loading buffer and boiled for 5 min. Thirty micrograms of protein (nuclear protein for NF-κB p65, plasma protein for ICAM-1) was loaded into each lane of 10% SDS-PAGE gel electrophoresis at 100 V for 4 h. After electrophoresis, the proteins were electroblotted onto NC membranes (Millipore, Bedford, MA, USA) at 9 V for 30 min. Nonspecific binding was blocked by incubation in phosphate-buffered saline (PBS) containing 0.1% Tween 20 (PBS-T) and 5% skim milk. The transferred membranes were incubated overnight at 4 °C with rabbit polyclonal antibodies NF-κB p65 and ICAM-1 (NF-κB p65 at 1:1 000 dilution, ICAM-1 at 1:500 dilution) against rat in PBS-T containing 5% skim milk. After washing thrice in PBS-T, the membranes were incubated with anti-rabbit IgG (Zhongshan Bio., China) conjugated to horseradish peroxidase at a dilution 1:2 000 in PBS-T containing 5% skim milk for 1 h at 37 °C. After three additional washes with PBS-T, the signals were visualized by DAB assay kit (Maixin-bio, China) and analyzed with a gel imaging system (Kodak system EDAS120).

Statistical analysis

All data were expressed as mean ± SD. Statistical analysis was performed using F- and Q-tests. $P < 0.05$ was considered statistically significant.

RESULTS

Lung pathological and BALF changes

The lung histological structure was normal in control group, while the lung tissues were obviously damaged with edema, hemorrhage, and inflammatory cell infiltration in I/R group. There was a significant difference between I/R and control group in pathological score ($P < 0.01$) and BALF content. After the administration of PDTC, the pathological score of lung injury and BALF content was improved significantly when compared to I/R group ($P < 0.05$, Table 1).

Serum IL-6 level

Compared to control group, serum IL-6 level was significantly increased in I/R group ($P < 0.01$,Table 2).

Table 1 Protein content of lung lavage fluid in different groups (mean±SD)

Groups	*n*	Lung lavage fluid (g/L)
Control group	8	0.8769 ± 0.1622
I/R group	8	1.2462 ± 0.3303[b]
PDTC group	8	0.9299 ± 0.2573[a]

[a]$P<0.05$ *vs* I/R group, [b]$P<0.01$ *vs* control.

Table 2 Serum IL-6 level in different groups (mean±SD)

Groups	*n*	IL-6 (ng/L)
Control group	8	22.51 ± 6.10
I/R group	8	42.85 ± 7.35[b]
PDTC group	8	28.08 ± 7.55[a]

[a]$P<0.05$ *vs* I/R group, [b]$P<0.01$ *vs* control.

Compared to I/R group, serum IL-6 level was significantly decreased in PDTC treatment group ($P<0.05$).

Lung MDA and MPO assay

Compared to control group, lung MDA and MPO significantly increased in I/R group ($P<0.01$). Compared to I/R group, lung MDA and MPO significantly decreased in PDTC treatment group ($P<0.01$, Table 3).

Immunohistochemical analysis of lung NF-κB and ICAM-1

The expression of NF-κB p65 and ICAM-1 in control group showed light brown immunostaining in cytoplasm and no staining in the nuclei. The significant positive expressions of NF-κB p65 and ICAM-1 as strong brown staining in cytoplasm and nuclei were observed in I/R group ($P<0.01$). Compared to I/R group, the positive rates of NF-κB p65 and ICAM-1 expression decreased significantly in PDTC group ($P<0.01$, Figures 1 and 2).

Western blot analysis of NF-κB and ICAM-1

Western blot showed weak NF-κB p65 and ICAM-1 positive signals in the lungs of control group. In contrast, significant increase of NF-κB p65 and ICAM-1 protein expression was found in I/R group ($P<0.01$). Compared to I/R group, the signals weakened significantly in PDTC group ($P<0.05$, Figure 3 and Table 4).

Table 3 Lung MDA and MPO level in different groups (mean±SD)

Groups	*n*	MDA (nmol/mg)	MPO (U/g)
Control group	8	1.44 ± 0.17	2.6075 ± 0.4372
I/R group	8	2.13 ± 0.39[d]	3.8763 ± 0.5682[d]
PDTC group	8	1.50 ± 0.18[b]	3.3350 ± 0.4712[a]

[a]$P<0.05$; [b]$P<0.01$ *vs* I/R group, [d]$P<0.01$ *vs* control.

Table 4 IOD level of lung NF-κB p65 and ICAM-1 in different groups (mean±SD)

Groups	*n*	IOD level	
		NF-κB p65	ICAM-1
Control group	8	36.295 ± 4.34	31.5 ± 6.87
I/R group	8	124.14 ± 21.22[b]	63.55 ± 18.45[b]
PDTC group	8	41.82 ± 9.16[a]	45.89 ± 8.86[a]

[a]$P<0.05$ *vs* I/R group, [b]$P<0.01$ *vs* control.

DISCUSSION

Previous studies have identified many mediators involved in the pathogenesis of lung injury induced by intestinal I/R[16,17], which exert their effects via a direct toxic action on lung tissue. Recent studies showed that these mediators such as NO, ROS, TNF-α, and ICAM-1 can be regulated by NF-κB. NF-κB is a rapid response transcription factor, which is maintained in the cytoplasm and consists of two subunits of 50 and 65 ku bound to an inhibitor protein, I-κB. This phosphorylated inhibitor unit is tagged by ubiquitin for subsequent proteolysis, and then the free NF-κB complex is able to translocate into the nuclei where it transactivates target genes[18,19]. As a consequence, activated polymorphonuclear neutrophils (PMNs) and pro-inflammatory cytokines (TNF-α, ILs) are released into the systemic circulation, interact with the vascular endothelium of distant organs, primarily the lungs, contributing to the systemic inflammatory response[20]. ICAM-1 is a member of the immunoglobulin superfamily, which is inducible by NF-κB and inflammatory cytokines such as IL-1β and TNF-α. ICAM-1 might be upregulated to involve the adhesion and infiltration of leukocytes into the injured site[21]. MPO resides in PMNs and its activity reflects the level of accumulation of PMNs.

In our study, 1 h of intestinal ischemia followed by 2 h of reperfusion induced lung injury manifested as a significant increase of BALF content and pathological injury score as well as PMN infiltration. These changes were parallel to the level of lung NF-κB p65, suggesting that the activation of NF-κB is involved in the pathogenesis of lung injury induced by intestinal I/R. As a consequence, activated PMNs and pro-inflammatory cytokines (such as IL-6) are released into the systemic circulation, and interact with the vascular endothelium of organs. Endothelial adhesion molecules expressed on the surface of endothelial cells (such as ICAM-1) play a key role in neutrophil chemoattraction, adhesion and emigration from the vasculature to the tissue, contributing to the systemic inflammatory response and organ injury[22,23]. The production of ROS, such as lung MDA and MPO (an index of tissue neutrophil count) was observed in I/R group. NF-κB upregulates neutrophil infiltration and expression of ICAM-1. PDTC, as an antioxidant, is involved in its ability to inhibit NF-κB[24-26] via the stabilization of I-κB-α[27] or via the inhibition of the ubiquitin–proteasome pathway[28]. In our study, PDTC reduced the neutrophil infiltration, lung expression of ICAM-1 and MPO activity, which can prevent the development of lung injury.

In conclusion, activation of NF-κB plays an important

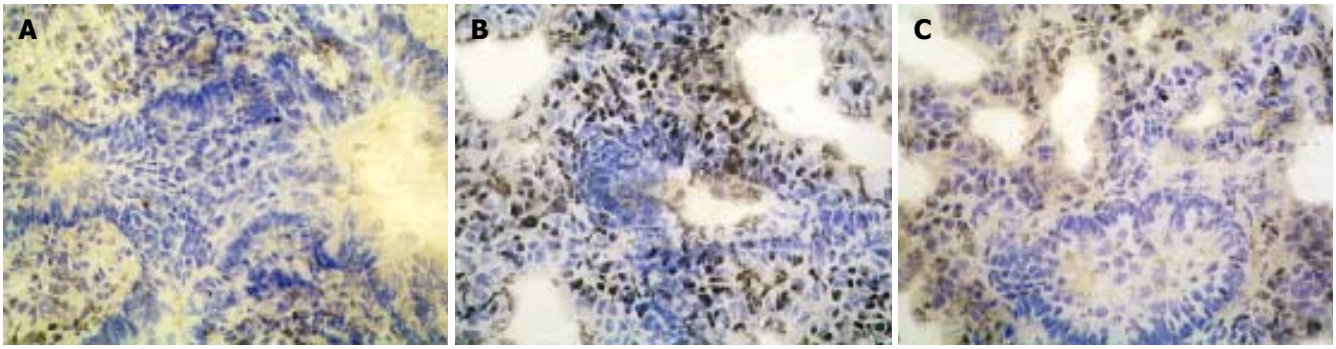

Figure 1 Expression of NF-κB p65 in lung tissue of control group (**A**), I/R group (**B**), and PDTC group (**C**).

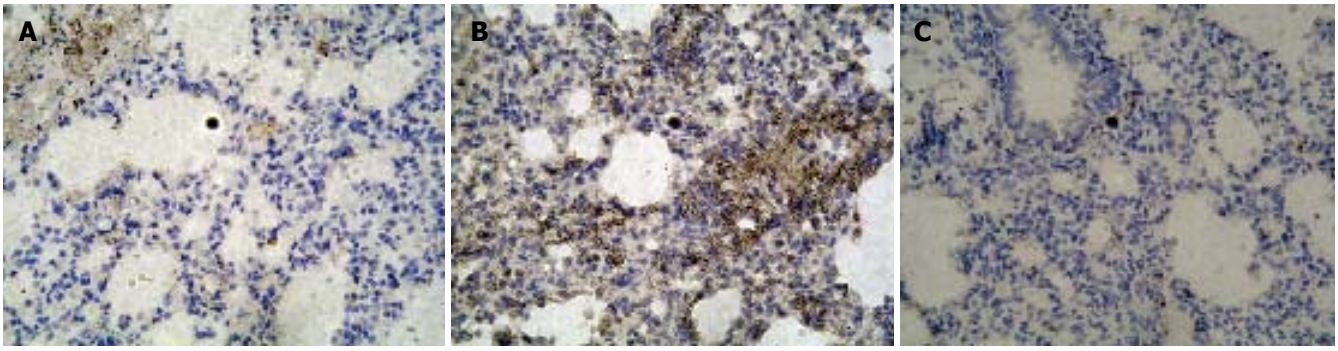

Figure 2 Expression of ICAM-1 in lung tissue of control group (**A**), I/R group (**B**), and PDTC group (**C**).

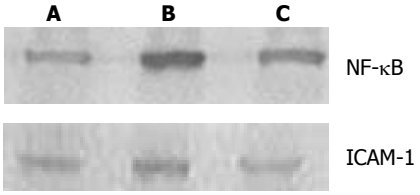

Figure 3 Lung NF-κB P65 and ICAM-1 protein signals from left to right side in control group (**A**), I/R group (**B**) and PDTC group (**C**). Compared to control group, the signals in I/R group increased significantly and weakened significantly in PDTC group.

role in the pathogenesis of lung injury induced by intestinal I/R by upregulating the neutrophil infiltration and lung ICAM-1 expression. PDTC can prevent lung injury induced by intestinal I/R by inhibiting the activity of NF-κB.

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REFERENCES

1 **Nüssler NC**, Müller AR, Weidenbach H, Vergopoulos A, Platz KP, Volk HD, Neuhaus P, Nussler AK. IL-10 increases tissue injury after selective intestinal ischemia/reperfusion. *Ann Surg* 2003; **238**: 49-58

2 **Pierro A**, Eaton S. Intestinal ischemia reperfusion injury and multisystem organ failure. *Semin Pediatr Surg* 2004; **13**: 11-17

3 **Antonsson JB**, Fiddian-Green RG. The role of the gut in shock and multiple system organ failure. *Eur J Surg* 1991; **157**: 3-12

4 **Hassoun HT**, Kone BC, Mercer DW, Moody FG, Weisbrodt NW, Moore FA. Post-injury multiple organ failure: the role of the gut. *Shock* 2001; **15**: 1-10

5 **Colletti LM**, Remick DG, Burtch GD, Kunkel SL, Strieter RM, Campbell DA. Role of tumor necrosis factor-alpha in the pathophysiologic alterations after hepatic ischemia/reperfusion injury in the rat. *J Clin Invest* 1990; **85**: 1936-1943

6 **Hu XM**, Lu Y, Yao SL. [Propofol reduces intercellular adhesion molecular-1 expression in lung injury following intestinal ischemia/reperfusion in rats]. *Zhongguo Weizhongbing Jijiu Yixue* 2005; **17**: 53-56

7 **Sun Z**, Andersson R. NF-kappaB activation and inhibition: a review. *Shock* 2002; **18**: 99-106

8 **Ghosh S**, May MJ, Kopp EB. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annu Rev Immunol* 1998; **16**: 225-260

9 **Barnes PJ**, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997; **336**: 1066-1071

10 **Hou J**, Baichwal V, Cao Z. Regulatory elements and transcription factors controlling basal and cytokine-induced expression of the gene encoding intercellular adhesion molecule 1. *Proc Natl Acad Sci U S A* 1994; **91**: 11641-11645

11 **Ledebur HC**, Parks TP. Transcriptional regulation of the intercellular adhesion molecule-1 gene by inflammatory cytokines in human endothelial cells. Essential roles of a variant NF-kappa B site and p65 homodimers. *J Biol Chem* 1995; **270**: 933-943

12 **Xu J**, Xie J, Bao M, Li Z, Yang Z. NF-kappaB/I-kappaB pathway during ischemia reperfusion injury of rat liver. *Chin Med J (Engl)* 2003; **116**: 1146-1149

13 **Megison SM**, Horton JW, Chao H, Walker PB. A new model for intestinal ischemia in the rat. *J Surg Res* 1990; **49**: 168-173

14 **Chiu CJ**, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch Surg* 1970; **101**: 478-483

15 **Cox G**. IL-10 enhances resolution of pulmonary inflammation in vivo by promoting apoptosis of neutrophils. *Am J Physiol* 1996; **271**: L566-L571

16 **Galili Y**, Ben-Abraham R, Weinbroum A, Marmur S, Iaina A, Volman Y, Peer G, Szold O, Soffer D, Klausner J, Rabau M, Kluger Y. Methylene blue prevents pulmonary injury after intestinal ischemia-reperfusion. *J Trauma* 1998; **45**: 222-25; discussion 222-25

17 **Chen ZB**, Zheng SS, Yuan G, Ding CY, Zhang Y, Zhao XH, Ni LM. Effects of intestinal lymph on expression of neutrophil adhesion factors and lung injury after trauma-induced shock. *World J Gastroenterol* 2004; **10**: 3221-3224

18 **Lee JI**, Burckart GJ. Nuclear factor kappa B: important transcription factor and therapeutic target. *J Clin Pharmacol* 1998; **38**: 981-993

19 **Kolesnick R**, Golde DW. The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. *Cell* 1994; **77**: 325-328

20 **Karin M**. The beginning of the end: IkappaB kinase (IKK) and NF-kappaB activation. *J Biol Chem* 1999; **274**: 27339-27342

21 **Mollà M**, Gironella M, Miquel R, Tovar V, Engel P, Biete A, Piqué JM, Panés J. Relative roles of ICAM-1 and VCAM-1 in the pathogenesis of experimental radiation-induced intestinal inflammation. *Int J Radiat Oncol Biol Phys* 2003; **57**: 264-273

22 **Olanders K**, Sun Z, Börjesson A, Dib M, Andersson E, Lasson A, Ohlsson T, Andersson R. The effect of intestinal ischemia and reperfusion injury on ICAM-1 expression, endothelial barrier function, neutrophil tissue influx, and protease inhibitor levels in rats. *Shock* 2002; **18**: 86-92

23 **Ishii H**, Ishibashi M, Takayama M, Nishida T, Yoshida M. The role of cytokine-induced neutrophil chemoattractant-1 in neutrophil-mediated remote lung injury after intestinal ischaemia/reperfusion in rats. *Respirology* 2000; **5**: 325-331

24 **Gukovsky I**, Gukovskaya AS, Blinman TA, Zaninovic V, Pandol SJ. Early NF-kappaB activation is associated with hormone-induced pancreatitis. *Am J Physiol* 1998; **275**: G1402-G1414

25 **Bowie A**, O'Neill LA. Oxidative stress and nuclear factor-kappaB activation: a reassessment of the evidence in the light of recent discoveries. *Biochem Pharmacol* 2000; **59**: 13-23

26 **Liu SF**, Ye X, Malik AB. In vivo inhibition of nuclear factor-kappa B activation prevents inducible nitric oxide synthase expression and systemic hypotension in a rat model of septic shock. *J Immunol* 1997; **159**: 3976-3983

27 **Virlos I**, Mazzon E, Serraino I, Di Paola R, Genovese T, Britti D, Thiemerman C, Siriwardena A, Cuzzocrea S. Pyrrolidine dithiocarbamate reduces the severity of cerulein-induced murine acute pancreatitis. *Shock* 2003; **20**: 544-550

28 **Si X**, McManus BM, Zhang J, Yuan J, Cheung C, Esfandiarei M, Suarez A, Morgan A, Luo H. Pyrrolidine dithiocarbamate reduces coxsackievirus B3 replication through inhibition of the ubiquitin-proteasome pathway. *J Virol* 2005; **79**: 8014-8023

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BASIC RESEARCH

Cocultivation of umbilical cord blood CD34$^+$ cells with retro-transduced hMSCs leads to effective amplification of long-term culture-initiating cells

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Abstract

AIM: To establish a novel coculture system for ex vivo expansion of umbilical cord blood(UCB) hematopoietic progenitors using thrombopoietin (TPO)/Flt-3 ligand (FL)-transduced human marrow-derived mesenchymal stem cells (tfhMSCs) as feeder.

METHODS: UCB CD34$^+$ cells were isolated and cultured using four culture systems in serum-containing or serum-free medium. Suitable aliquots of cultured cells were used to monitor cell production, clonogenic activity, and long-term culture-initiating culture (LTC-IC) output. Finally, the severe-combined immunodeficient (SCID) mouse-repopulating cell (SRC) assay was performed to confirm ability of the cultured cells to reconstitute long-term hematopoiesis.

RESULTS: There were no significant differences in the number of total nucleated cells among different culture systems in serum-containing medium during 21-d culture. However, on d 14, the outputs of CD34$^+$ cells, CFU-C and CFU-GEMM in tfhMSCs coculture system were significantly enhanced. LTC-IC assay demonstrated that the tfhMSCs coculture system had the most powerful activity. The severe-combined immunodeficient (SCID) mouse repopulating cell (SRC) assay confirmed extensive ability of the expanded cells to reconstitute long-term hematopoiesis. Furthermore, PCR analysis demonstrated the presence of human hematopoietic cells in the bone marrow and peripheral blood cells of NOD/SCID mice.

CONCLUSION: The TPO/FL-transduced hMSCs, in combination with additive cytokines, can effectively expand hematopoietic progenitors from UCB in vitro and the tfhMSCs coculture system may be a suitable system for ex vivo manipulation of primitive progenitor cells under contact culture conditions.

Key words: Mesenchymal stem cells; Thrombopoietin; Flt-3 ligand; Hematopoiesis

Xie CG, Wang JF, Xiang Y, Qiu LY, Jia BB, Wang LJ, Wang GZ, Huang GP. Cocultivation of umbilical cord blood CD34$^+$ cells with retro-transduced hMSCs leads to effective amplification of long-term culture-initiating cells. *World J Gastroenterol* 2006; 12(3): 393-402

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INTRODUCTION

Hematopoietic stem cells (HSCs) are generally defined as cells having the self-renewing potential and the capacity to give rise to differentiated cells of all hematopoietic lineages[1]. Therefore, HSC transplantation is performed for complete healing of hematologic disorders and as a supportive therapy after high-dose chemotherapy against malignant diseases. HSCs can be collected from peripheral blood (PB), bone marrow (BM), and umbilical cord blood (UCB). Human UCB is thought to contain a high number of primitive hematopoietic cells, because the number of severe combined immunodeficiency (SCID)-repopulating cells (SRCs) in nonobese diabetic/SCID (NOD/SCID) mice that had received transplants from UCB was higher than that in NOD/SCID mice that had received transplants from other sources[2-4]. Moreover, the frequency of graft-versus-host disease, which is a severe side effect of HSC transplantation in patients, is reduced among patients receiving transplants from UCB[5], and UCB can be obtained from the cord-blood bank network. However, the total number of UCB HSCs harvested from one donor's UCB is limited and is not sufficient for HSC transplantation in an adult patient. To overcome this problem, attention has been increasingly focused on

ex vivo expansion of HSCs. Many approaches have been reported during the last decade, and they can be divided into 2 categories. The first category is treatment of HSCs with various combinations of cytokines. Treatment with the following combinations of cytokines increased the progenitor/stem cell population by 2- to 30-fold in the relatively short period of 10 to 14 d: Flt-3 ligand (FL), stem cell factor (SCF), and thrombopoietin (TPO); SCF, granulocyte-colony stimulating factor (G-CSF), and megakaryocyte growth and development factor (MGDF); FL, SCF, G-CSF, interleukin-3 (IL-3), and interleukin-6 (IL-6); and FL, SCF, and IL-6[6-9]. However, it is difficult to maintain HSC activity in long-term cultures even if the total number of hematopoietic cells could be expanded. Hence, these methods could be improved for use in clinical settings. The second category involves using stromal cells. It has been reported that the SCID-repopulating activity (SRA) of human HSCs could be maintained by coculture with murine stromal cells for 7 wk[10], and that the SRA could be maintained by coculture with the AGM-S3 stromal cell line for 4 wk[11]. MS-5 expanded SRCs for 2 wk[12]; FBMD-1 expanded cobblestone area-forming cells by 90-fold[13]; HESS-5 expanded SRCs for only 5 d[14,15]. Contact between HSCs and stromal cells is important for maintaining the function of HSCs[16,17]. However, when human HSCs are cocultured with nonhuman stromal cells, the expanded human HSCs might have a risk of being exposed to an unknown viral contamination in animal stromal cells.

Several methods of ex vivo expansion using human primary stromal cells were reported[18,19]. When HSCs were cocultured with human primary stromal cells, the HSCs were expanded for 2 to 4 wk. However, in general, when human primary somatic cells divide in an in vitro culture, the telomeric DNA at the end of the chromosome shortens at each cell division. Then, the replication of human primary cells slows (aging occurs), and the cells finally cease to divide (crisis phase)[20,21]. To obtain a sufficient number of primary stromal cells for use on a clinical scale, we have to harvest BM many times, and we cannot ignore the burden on the donor. To solve this problem, trials to establish human stromal cell lines using transduction of viral antigens such as human papillomavirus (HPV) E6/E7 and simian virus 40 (SV40) large T have been reported[22-24]. These stromal cells could maintain HSCs, but the possibility of transformation was mentioned[25].

Recently, it was shown that Mesenchymal stem/progenitor cells in human UCB and placenta could support ex vivo expansion of $CD34^+$ hematopoietic stem cells[26,27]. However, no report has investigated whether transduced human marrow-derived mesenchymal stem cells (hMSCs) could be useful in ex vivo expansion of UCB hematopoietic progenitors. Previous research has shown that TPO and FL, the two early-acting cytokines, could lead to significant expansion of HSC populations, including long-term culture-initiating cells (LTC-IC). Our group have introduced TPO and FL genes into cultured hMSCs by retroviral vector transfer and demonstrated long-term expression in vitro and in vivo. In this study, we attempted to establish a coculture system for ex vivo expansion of UCB hematopoietic progenitors using TPO/FL-transduced hMSCs (tfhMSCs) as feeder. As a result, tfhMSCs is capable of expanding UCB hematopoietic cells in synergy with extra cytokines *in vitro*.

MATERIALS AND METHODS

Analysis of expression of the TPO and FL gene by enzyme-linked immunosorbent assay

Untransduced and TPO/FL-transduced hMSCs (tfhMSCs) were cultured in minimal essential medium α (MEM-α; HyClone, Logan, UT, USA) supplemented with 100 mL/L fetal bovine serum (FBS; GibcoBRL, GrandIsland, NY, USA) at 37 °C with 50 mL/L CO_2 in humidified air. For assaying TPO and FL secretion, untransduced hMSCs and/or tfhMSCs were passaged when cells reached 90% confluence by transferring 2.5×10^6 to 5.0×10^6 cells into a 75-cm^2 flask with 12 mL of hMSCs medium. Twenty-four hours later, 1 mL of culture supernatant was collected and stored at -80 °C. The assay was performed in triplicate using the TPO and FL ELISA kit (BioSource International, Camarillo, CA, USA). The level of TPO and FL was normalized to the level of endogenously expressed IL-6 measured with an IL-6 ELISA kit (BioSource International) using the procedures suggested by manufacturer. Plates were read on a microplate reader (Bio-Rad Laboratories, Hercules, CA, USA) and the data were analyzed using Microsoft Excel.

UCB sample collection and $CD34^+$ cell purification

UCB were collected from normal full-term pregnancies according to the regulations of the Research Ethics Committee of Women's Hospital, School of Medicine Zhejiang University. Mononuclear cells (MNC) were isolated using Ficoll-Hypaque (1.077 ± 0.001 Kg/L, Sigma, St. Louis, MO), washed, and resuspended in Iscove's modified Dulbecco's medium (IMDM; HyClone, Logan, UT) supplemented with 100 mL/L fetal bovine serum (FBS; GibcoBRL, GrandIsland, NY). $CD34^+$ cell purification utilized positive selection using the *miniMACS* immunomagnetic separation system (Miltenyi Biotec GmbH, Glodbach, Germany) according to the manufacturer's instructions. Briefly, MNCs were suspended in buffer containing phosphate-buffered saline (PBS), 5 mL/L bovine serum albumin (BSA; Sigma), and 2 mmol/L EDTA (BSA-EDTA-PBS), and incubated for 15 min with monoclonal hapten-conjugated anti-CD34 antibody (clone: QBEND/10) and human Ig to prevent nonspecific binding. Washed cells were resuspended in BSA-EDTA-PBS and incubated for 15 min with colloidal super-paramagnetic microbeads conjugated to an anti-hapten antibody. After labeling, the cell suspension was passed through a column (VS^+ separation column) held within a magnetic field causing $CD34^+$ cells to be retained in the column. $CD34^+$ cells were collected by removal of the column from the magnet and washing with BSA-EDTA-PBS. Ninety-six percent or more of the enriched cells were $CD34^+$ by flow cytometric analysis.

Human cytokines

Recombinant human TPO, granulocyte-macrophage

colony-stimulating factor (GM-CSF), and erythropoietin (EPO) were purchased from Peprotech (London, UK). IL-3 and IL-6 was purchased from RELIATech GmbH (Braunschweig, Germany). Recombinant human SCF was a gift from Amgen Biologicals (Thousand Oaks, CA). Recombinant human FL was purchased from R&D Systems (Minneapolis, MN). The final concentrations of cytokines were as follows: TPO, 50 μg/L; FL, 50 μg/L; IL-3, 20 μg/L; IL-6, 20 μg/L; SCF, 50 μg/L; GM-CSF, 10 μg/L; and EPO, 3 000 U/L.

Culture systems

Stroma-free culture and coculture with tfhMSCs or hMSCs were performed in culture media in 24-well microplates (Costar, Bethesda, MD). Serum-containing liquid culture was carried out using a medium containing 125 mL/L horse serum (HS; HyClone), 125 mL/L FBS, 10^{-4} mol/L 2-mercaptoethanol (Sigma), 2 mmol/L L-glutamine (Sigma) and IMDM supplemented with 10^{-6} mol/L hydrocortisone (Sigma) with or without feeder layer. In the coculture, tfhMSCs or hMSCs were seeded at 1×10^5 cells per well with MEM-α supplemented with 100 mL/L FBS. After obtaining a confluent feeder layer, cells were washed five times and subjected to γ-irradiation at a dose of 12 Gy. the medium was then changed for coculture. Totally 20 000 UCB CD34$^+$ cells were expanded for 21 d under four conditions: 1) tfhMSCs coculture system (tfhMSCs + SCF + IL-3 + IL-6 + GM-CSF); 2) hMSCs coculture system (hMSCs + TPO + FL + SCF + IL-3 + IL-6 + GM-CSF); 3) cytokines culture system (TPO + FL + SCF + IL-3 + IL-6 + GM-CSF); 4) hMSCs (TPO/FL-free) culture system (hMSCs + SCF + IL-3 + IL-6 + GM-CSF). On d 7 and 14 of culture, the medium in each well was removed and replaced with fresh medium. On d 7, 14 and 21 of culture, aliquots of cultured cells were harvested and subjected to cell count, clonal cell culture, and flow cytometric analysis when contamination of stromal cells in the harvested cells was negligible (< 2%) by microscopic visualization. On d 14, cultured cells were harvested and subjected to LTC-IC assay and SRC assay. Short-term (7 d) serum-free liquid culture was carried out using StemPro™-34SFM (GibcoBRL) supplemented with StemPro™-34 Nutrient Supplement (GibcoBRL), 2 mmol/L L-glutamine, and penicillin/streptomycin (GibcoBRL).

Immunophenotyping by flow cytometry

Aliquots of cells were suspended in EDTA-BSA-PBS and incubated with mouse IgG (InterCell Technologies, Hopewell, NJ) to block nonspecific binding. Cells were then reacted for 15 min with FITC- and PE-conjugated monoclonal antibodies at 4℃. Unbound antibodies were removed by two washes, and cells were resuspended in EDTA-BSA-PBS. Stained cells were then passed through a nylon mesh filter and subjected to two-color flow cytometric analysis. Cells labeled with FITC- and PE-conjugated mouse isotype-matched antibodies were used as negative controls. The analysis was performed using an FACsort flow cytometer (Becton Dickinson, San Jose, CA) with CELLQUESTTM software (Becton Dickinson). At least 10 000 events were acquired for each analysis. Antibodies used were as follows: FITC-conjugated CD14, CD15, CD19, CD33, CD34, and CD41; PE-conjugated CD38 and CD45 antibodies. Glycophorin A antibodies were from Immunotech (Marseille, France). CD14, CD33, and CD45 antibodies were from Pharmingen (San Diego, CA) and all others were from Becton Dickinson. Furthermore, in some experiments, aliquots of cultured cells were subjected to three-color flow cytometric analysis to assess the lineage commitment of progenitors. Samples were incubated for 15 min with biotin-conjugated anti-CD34 (Immunotech, Marseille, France). Cells labelled with a biotin-conjugated mouse isotype-matched antibody were used as a negative control. After washing, cells were labeled with streptavidin PerCP (Becton Dickinson), PE-conjugated anti-CD38, and various FITC-conjugated monoclonal antibodies. Three-color flow cytometry was performed using an FACSCalibur (Becton Dickinson) with CellQuest software (Becton Dickinson).

Colony-forming cell assay (CFC assay)

Aliquots from initial UCB samples or cultured cells were incubated in methylcellulose media at concentrations of $1\text{-}2\times10^5$ cells/L for purified CD34$^+$ cells and $5\text{-}10\times10^5$ cells/L for cultured cells in 35-mm tissue culture dishes (Costar). One milliliter of culture mixture contained 12 mL/L 1500 cp methylcellulose (Sigma), MEM-α, 10 g/L deionized fraction Ⅴ BSA (Sigma), 10^{-4} mol/L 2-mercaptoethanol, 300 mL/L fetal calf serum (JRH Biosciences, Lenexa, KS), EPO, IL-3, SCF, GM-CSF, and cells. Dishes were incubated at 37 °C in a humidified atmosphere with 50 mL/L CO_2 in air. All cultures were done in triplicate. Total colony-forming units in culture (CFU-C) and mixed colonies containing erythroid and myeloid cells and megakaryocytes (CFU-GEMM) consisting of 50 or more cells were scored under an inverted microscope at 21 d of culture. To assess the accuracy of in situ identification, individual colonies were lifted with an Eppendorf micropipette under direct microscopic visualization, spread on glass slides using a cytocentrifuge and studied with May-Grunwald-Giemsa staining.

Long-term culture-initiating cell assay (LTC-IC assay)

LTC-IC assay was performed as described by Sutherland *et al.*[28], with slight modifications. Briefly, bone marrow stromal cells derived from hematologically normal donors were seeded at 10^5 cells per well in 96-well flat-bottomed plates (Costar) with MEM-α supplemented with 100 mL/L FBS. After obtaining semiconfluent feeder layers, stromal cells were irradiated with 15-Gy using a ^{60}Co γ-irradiator. CD34$^+$ cell subpopulations purified from UCB or those isolated from cultured cells by sorting with an FACSVantage (Becton Dickinson) were seeded at limiting dilution on the feeder layer in serum-containing media. For each evaluation, at least three cell concentrations were used with 24 replicates per concentration. Culture plates were incubated at 37 °C with 50 mL/L CO_2 in air and weekly changes of medium. After 5 wk of culture, cells were assayed for CFU-C in methylcellulose medium. Colonies were scored 2 wk later. The frequency of wells in which there were no clonogenic progenitors was determined

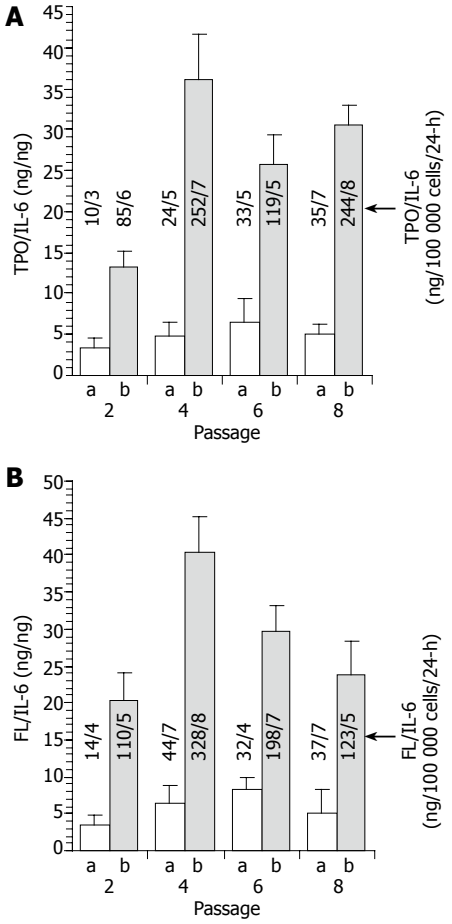

Figure 1 Maintenance of TPO and FL production *in vitro*. TPO (**A**) and FL (**B**) levels secreted from tfhMSCs and untransduced hMSCs and endogenous IL-6 levels were assayed in the medium after 2, 4, 6, and 8 passages in culture. The level of TPO and FL in the supernatant was normalized to the level of endogenous IL-6 and the ratio was plotted for passage number. In addition, the absolute values (ng/100 000 cells/24 h) of TPO, FL and IL-6 are shown (arrow). Data were obtained from triplicate ELISA measurements. a = untransduced hMSCs; b = tfhMSCs.

according to the number of the initial input population. Poisson statistics were applied to the single-hit model and the frequency of LTC-IC was calculated with the maximum likelihood estimator.

SCID-repopulating cells assay (SRC assay)

SRC assay was performed as previously described[29], with slight modifications. Briefly, 8-wk-old male NOD/Shi-scid (NOD/SCID) mice were obtained from the Central Institute for Experimental Animals, Shanghai Institutes for Biological Sciences, CAS. All animals were handled under sterile conditions and maintained under microisolators in the animal facility located at Zhejiang Academy of Medical Sciences. Human hematopoietic cells at the indicated doses were transplanted by tail-vein injection into sublethally irradiated mice (350 cGy using a linear accelerator). Cells were co-transplanted with irradiated (15 Gy using a ^{60}Co γ-irradiator) nonrepopulating CD34$^-$ cells as accessory cells. Mice were killed 7 wk after transplantation, and the bone marrow (from the femurs and tibiae) and peripheral blood cells (from the retro-orbital venous plexus using heparin-coated micropipettes) were harvested. The presence of human hematopoietic cells was determined by detection of cells positively stained with FITC-conjugated antihuman CD45 using flow cytometry. Polymerase chain reaction (PCR) analysis using human Alu sequence primers (5' - GTGGGCGACAGAACGAGATTCTAT; 5' - CTCACTACTTGGAGACAGGTTCA) was also performed to confirm flow cytometric results.

Statistical analysis

Results are expressed as mean ± SD. Statistical comparisons were performed using the two-sided Student's *t*-test. Iterative approximation of Newton' method was performed using Microsoft Visual Basic 6.0 software.

RESULTS

Analysis of expression of TPO and FL gene from tfhMSCs and untransduced hMSCs

We transduced hMSCs with the secreted cytokine TPO and FL, which allows direct quantitation of the extracellular product. Human MSCs were transduced with a retroviral vector (pLXINTF) expressing TPO, FL and neomycin followed by selection in the presence of G418 for 2 wk. Cultures were maintained and expanded further for up to 8 passages. Twenty-four hours after each replating aliquots of supernatant from tfhMSCs and untransduced hMSCs was removed for measurement of secreted TPO and FL. The TPO and FL secretion value obtained was normalized to the secretion value obtained for endogenous human IL-6 measured in the same sample aliquot. The absolute values for each cytokine are shown in Figure 1. We chose IL-6 as the control cytokine because expression was in a range similar to that of the transduced gene product and previous data showed similar expression of IL-6 from hMSCs over time in hMSC cultures[30].

We observed TPO transgene expression from tfhMSCs and untransduced hMSCs averaging 175 ± 85 and 25 ± 11 ng/10^{5}cells/24 h respectively (Figure 1A) and FL transgene expression averaging 190 ± 99 and 31 ± 12 ng/10^5 cells/24 h respectively (Figure 1B); Endogenous IL-6 protein levels averaging 6 ± 2 ng/10^{5}cells/24 h. The cytokine ratio (transduced/endogenous) of tfhMSCs demonstrated a 14- to 36-fold and 22- to 41-fold increase respectively in TPO and FL secretion over endogenous IL-6 expression. The results demonstrate that in vitro transgene expression from tfhMSCs was maintained for at least three months in culture.

Assessment of the supportive effects of tfhMSCs on proliferation of UCB primitive progenitor cells (PPC) in synergy with extra cytokines

To determine whether tfhMSCs was capable of supporting ex vivo expansion of UCB-derived hematopoietic cells, four culture systems were established as shown in materials and methods. In the stroma-containing culture, tfhMSCs or untransduced hMSCs were irradiated and cocultured with CD34$^+$ cells from UCB, and in the stroma-free culture only cytokines were used.for expansion. The total number of nucleated cells, CD34$^+$ cells, CFU-C

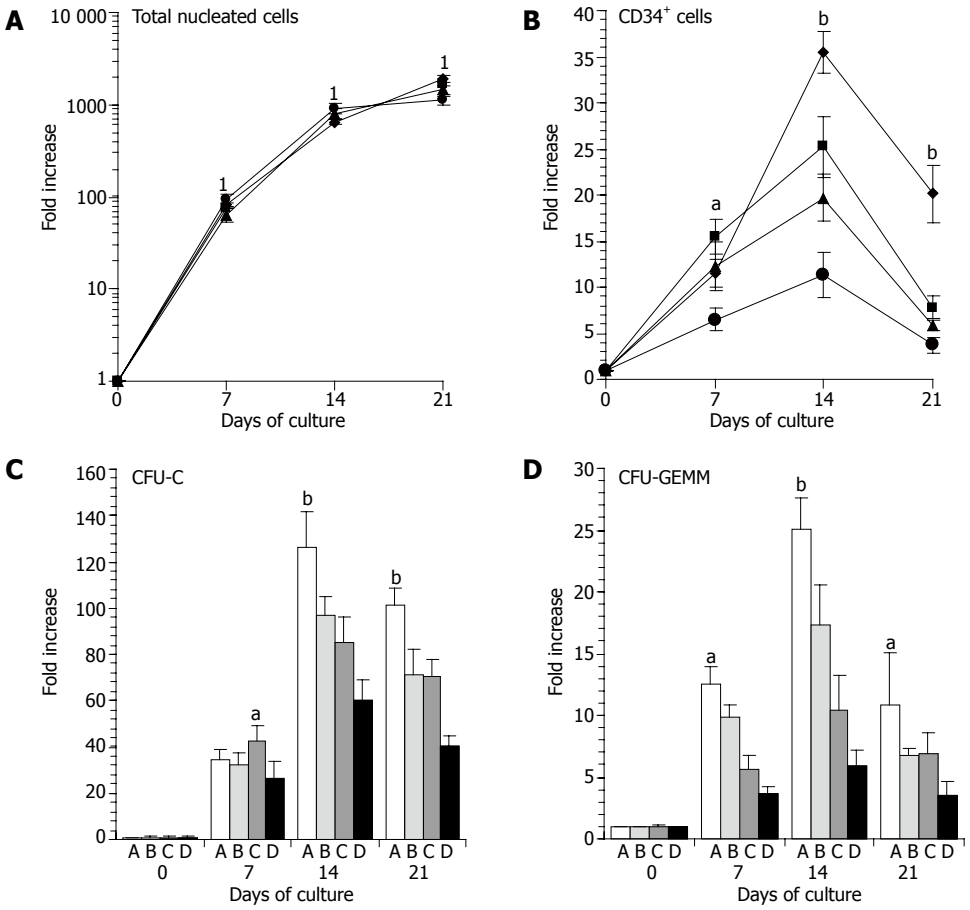

Figure 2 Effects of several culture systems on ex vivo expansion of hematopoietic progenitors. UCB CD34$^+$ cells were cultured in serum-containing medium using four culture systems. **A:** Total nucleated cells; **B:** CD34$^+$ cells; **C:** Total colony-forming units in culture (CFU-C); **D:** Mixed colonies containing erythroid and myeloid cells and megakaryocytes (CFU-GEMM). The results represent the mean fold increase ± SD of three different experiments on d 7, 14, and 21 of culture. [1]No significant differences between different culture systems. [a]$P < 0.05$, [b]$P < 0.01$ as compared with other three groups. ◆= tfhMSCs coculture system; ■= hMSCs coculture system; ▲ = cytokines culture system; ● = hMSCs (TPO/FL-free) culture system. A = tfhMSCs coculture system; B=hMSCs coculture system; C = cytokines culture system; D = hMSCs (TPO/FL-free) culture system.

and CFU-GEMM was evaluated on d 7, 14 and 21. CD34 and CD38 expression among hematopoietic cells in four culture conditions were also determined on d 14.

We first assessed the effect of tfhMSCs on ex vivo expansion of UCB-PPC in the serum-containing culture. As a result, tfhMSCs alone could not effectively support proliferation of UCB hematopoietic progenitors (data not shown). The effects of four culture systems on ex vivo expansion of total nucleated cells and CD34$^+$ cells were then studied in serum-containing medium. During culture, there were no significant differences in the number of total nucleated cells among different culture systems (Figure 2A).The expansion magnitude of CD34$^+$ cells by tfhMSCs coculture system (11.52 ± 1.51 fold) and cytokines culture system (12.32 ± 2.69 fold) was lower than that by hMSCs coculture system (15.40 ± 1.89 fold) on d 7 ($P < 0.05$), and hMSCs (TPO/FL-free) culture system (6.59 ± 1.26 fold) manifested the lowest expansion capacity ($P < 0.01$) among the four groups (Figure 2B). However, on d 14, CD34$^+$ cells were generated more by the tfhMSCs coculture system (35.42 ± 2.25 fold) than by hMSCs coculture system (25.24 ± 3.32 fold), cytokines culture system (19.75 ± 2.56 fold) and hMSCs (TPO/FL-free) culture system (11.32 ± 2.48 fold) ($P < 0.01$)(Figure 2B). Although fold increase of CD34$^+$ cells was largely decreased among all the four systems on d 21, the expansion magnitude of CD34$^+$ cells by tfhMSCs coculture system (20.15 ± 3.16 fold, $P < 0.01$) was higher than that by the three systems (Figure 2B).

Next, we determined the outputs of CFU-C and CFU-GEMM. The expansion magnitude of CFU-C in tfhMSCs coculture system (126.54 ± 15.42 fold), although lower than cytokines culture system on d 7 ($P < 0.05$), was much higher than other three culture systems on d 14 ($P < 0.01$). Interestingly, tfhMSCs coculture system significantly stimulated production of CFU-C on d 21 (101.12 ± 7.23 fold, $P < 0.01$) (Figure 2C). The output of CFU-GEMM in tfhMSCs coculture system on d 7, 14 and 21 was also enhanced (12.65 ± 1.36 fold, 25.16 ± 2.53 fold and 10.93 ± 4.12 fold, respectively) ($P < 0.01$) (Figure 2D). Analysis for expression of CD34 and CD38 by flow cytometry among the four culture systems on d 14 was shown in Figure 3.

Ex vivo expansion of UCB CD34$^+$ cells in a short-term (7 d) serum-free tfhMSCs coculture system

The synergistic effects of tfhMSCs and additive cytokines

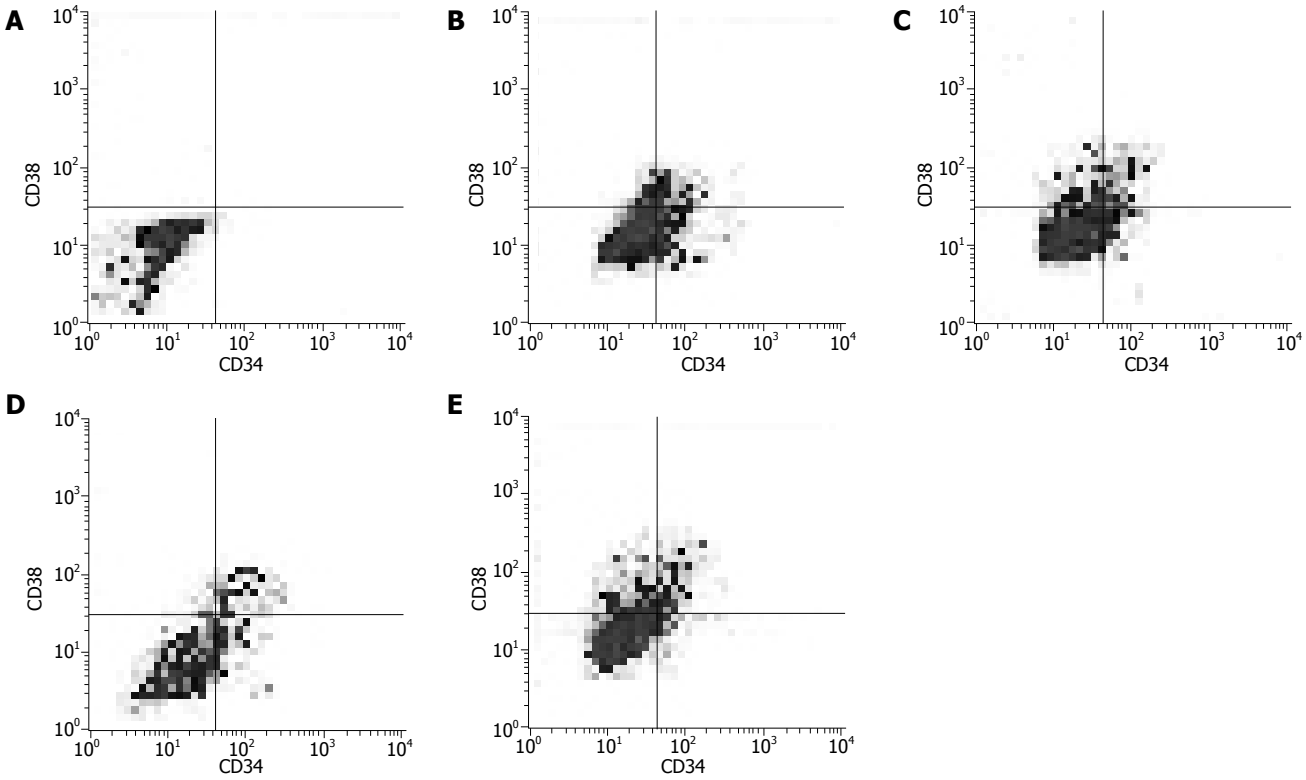

Figure 3 Comparison of CD34 and CD38 expression among hematopoietic cells in different culture systems. $CD34^+$ cells derived from a single delivery were cultured using different culture systems in serum-containing medium. On d 14 of culture, aliquots of harvested cells were subjected to flow cytometric analysis. **A** = Negative control; **B** = tfhMSCs coculture system; **C** = hMSCs coculture system; **D** = cytokines culture system; **E** = hMSCs (TPO/FL-free) culture system.

on ex vivo expansion of UCB hematopoietic progenitors was also studied in serum-free culture for a short duration. After 7 d of culture, the number of total nucleated cells in tfhMSCs coculture system was remarkably increased ($P<0.01$)(Table 1). As a result, the mean number of total nucleated cells was approximately 50 to 90 times the initial input number. $CD34^+$ cells were generated more by tfhMSCs coculture system than by other three groups ($P<0.01$); the number of $CD34^+$ cells was over 12 times the initial input number. The outputs of CFU-C and CFU-GEMM in tfhMSCs coculture system were also increased approximately 40-fold ($P<0.05$) and 15-fold ($P<0.01$), respectively.

LTC-IC assay using the $CD34^+$ population isolated from cells cultured in four culture systems

To determine whether cells generated in four culture systems could preserve the ability to sustain long-term hematopoiesis, the LTC-IC frequency in cells cultured by these systems was quantified. Initially, isolated UCB $CD34^+$ cells were cultured for 14 d using four culture systems in the serum-containing culture. Cultured cells were harvested and subjected to a second $CD34^+$ cell purification by sorting. LTC-IC assay was performed using sorted $CD34^+$ cell populations, as well as those initially prepared from UCB (control samples). The LTC-IC frequency was determined as previously described[28]. As shown in Table 2, Although the yields of LTC-IC expansion in hMSCs coculture system (5.32 ± 1.73 fold) was higher than cytokines culture system (3.58 ± 1.48 fold) and hMSCs (TPO/FL-free) culture system (2.79 ± 0.56 fold), the tfhMSCs coculture system demonstrated the most powerful activity (10.23 ± 2.89 fold, $P<0.01$). The findings suggest that the tfhMSCs coculture system might be a novel as well as efficient culture system for UCB-derived hematopoietic progenitor cells.

Effects of coculture system using tfhMSCs as feeder layer on human reconstituting hematopoietic progenitors

Accordingly, we studied the SRC assay to determine whether cells cultured in the tfhMSCs coculture system were capable of long-term multilineage reconstitution *in vivo*. Purified 1×10^5 UCB $CD34^+$ cells were initially cultured for 14 d using the tfhMSCs coculture system; harvested cells were then transplanted into NOD/SCID mice. As controls, uncultured 100 000 UCB $CD34^+$ cells obtained from the same sources were also transplanted into other mice. Seven weeks after transplantation, human $CD45^+$ cells were found in the bone marrow, and peripheral blood cells of mice transplanted with the cultured cells (Figure 4A, and data not shown). There were marked differences in the percentage of chimerism between bone marrow cells in mice transplanted with cultured cells and those transplanted with control samples. Human $CD45^+$ cells in the murine bone marrow were further subjected to flow cytometric analysis to determine multilineage reconstitution. As a result, human $CD45^+$ cells were positive for CD34, CD33, CD14, CD41, glycophorin A, or CD19 (Figure 4B). Furthermore, PCR analysis demonstrated the presence of human hematopoietic cells in the bone marrow and peripheral blood cells of NOD/SCID mice (Figure 5).

Table 1 Evaluation of effects of four culture systems on ex vivo expansion of hematopoietic progenitors in short-term serum-free culture

Culture systems	Total nucleated cells	CD34$^+$ cells	CFU-C	CFU-GEMM
tfhMSCs coculture system	87.56±9.51[b]	12.39±2.34[b]	40.59±6.23[a]	15.84±3.96[b]
hMSCs coculture system	53.23±6.79[a]	9.87±2.89[a]	38.78±4.28[a]	10.57±2.58[b]
Cytokines culture system	46.25±4.89	9.58±1.26[a]	27.68±3.32	7.19±1.96
hMSCs (TPO/FL-free) culture system	48.52±6.49	6.79±0.62	32.85±4.35	5.67±1.09

[a]$P<0.05$, [b]$P<0.01$ *vs* as compared with hMSCs (TPO/FL-free) culture system.

Table 2 Results of LTC-IC assay using UCB CD34+ cells or those generated by four culture systems

Culture systems	LTC-IC frequency		Fold LTC-IC amplification
	Pre-expansion	Post-expansion	
hMSCs (TPO/FL-free) culture system	1/619.57±120.89	1/102 258.56±9 568.49	2.79±0.56
Cytokines culture system	1/625.38±154.26	1/84 112.78±10 867.64	3.58±1.48
hMSCs coculture system	1/621.76±138.59	1/93 773.42±20 346.23	5.32±1.73
tfhMSCs coculture system	1/617.43±119.76	1/24 140.96±7 586.62	10.23±2.89[b]

[b]$P<0.01$ *vs* calculated by the Student's *t*-test, when data of the tfhMSCs coculture system were compared with data in the other groups.

DISCUSSION

In this study we demonstrated that TPO/FL–transduced human marrow-derived mesenchymal stem cells (tfhMSCs) could effectively support ex vivo expansion of UCB-PPC in synergy with human cytokines. We assessed the supportive effects of four culture systems on proliferation of PPC in serum-containing liquid medium. Although there were no significant differences in the number of total nucleated cells among the different culture systems during culture, tfhMSCs coculture system could dramatically enhance generation of CFU-C, CFU-GEMM and CD34$^+$ cells, and more importantly was capable of expanding LTC-IC.

In ex vivo expansion of human UCB-PPC, there are several benefits to using the tfhMSCs as the feeder: (1) tfhMSCs can be maintained easily; (2) consistent hematopoietic-supportive effects are repeatedly obtained; (3) additive TPO and FL are not needed in culture system. The effects of tfhMSCs coculture system were comparable to those obtained by classical culture, including cytokines culture and contact culture. Although contamination of tfhMSCs into cultured cells is a problem, and it is difficult to harvest cultured hematopoietic cells completely, since a number of cultured cells migrate under feeder layers, the tfhMSCs coculture system was considered to be a suitable system for ex vivo manipulation of PPC under stroma-contact culture conditions. Moreover, the tfhMSCs, unlike the primary stromal cells[17,18], could be expanded and cryopreserved without transformation. Thus, we can prepare a large quantity of these human tfhMSCs at any time. Taking advantage of this culture system, clinical research on ex vivo expansion could be facilitated. For example, progenitor cells such as CD34$^+$ cells and CFU-C were extensively expanded more than 125-fold in this system in 2 wk, and it may be possible to use these expanded cells as a new source of blood transfusion after differentiation of the expanded cells into megakaryocytes or erythroblast progenitor cells[31].

Although the present study and others[5,8,32] have demonstrated that addition of TPO and FL in culture systems could lead to significant expansion of HSC populations, including LTC-IC, the tfhMSCs coculture system, which have no addition of TPO and FL, manifested higher expansion of LTC-IC. The mechanism of supportive effect of tfhMSCs remains unknown. Previous investigations have shown that the very hematopoietic cell types whose maximum proliferation in vitro depends on stimulation by the highest concentrations of cytokines[32]. The ability that tfhMSCs expressing TPO and FL can maintain a higher concentrations of TPO and FL in medium may be a reason.

Serum-free culture could prevent UCB primitive-cell differentiation[14], and when a serum-free medium is used, the risks of contamination of heterogenic antigen and infectious danger were small[33]. We then assessed the supportive effects of four culture systems on proliferation of PPC in serum-free liquid medium. We attempted ex vivo manipulation for a short duration. As a result, adequate expansion of UCB-PPC was obtained in tfhMSCs coculture system. Furthermore, compared with serum-containing culture the number of CD34$^+$ cells was not increased and the total nucleated cells were not significantly decreased during culture; However, the output of CFU-C and CFU-GEMM was enhanced, suggesting that serum-free conditions prevent primitive-cell differentiation.

Previous findings showed that UCB LTC-IC were present among the CD34$^+$ cell fraction[34,35]. Furthermore, Bhatia *et al.* identified the SRC that were capable of multilineage reconstitution of human hematopoiesis in the bone marrow of NOD/SCID mice[29]. Therefore, the

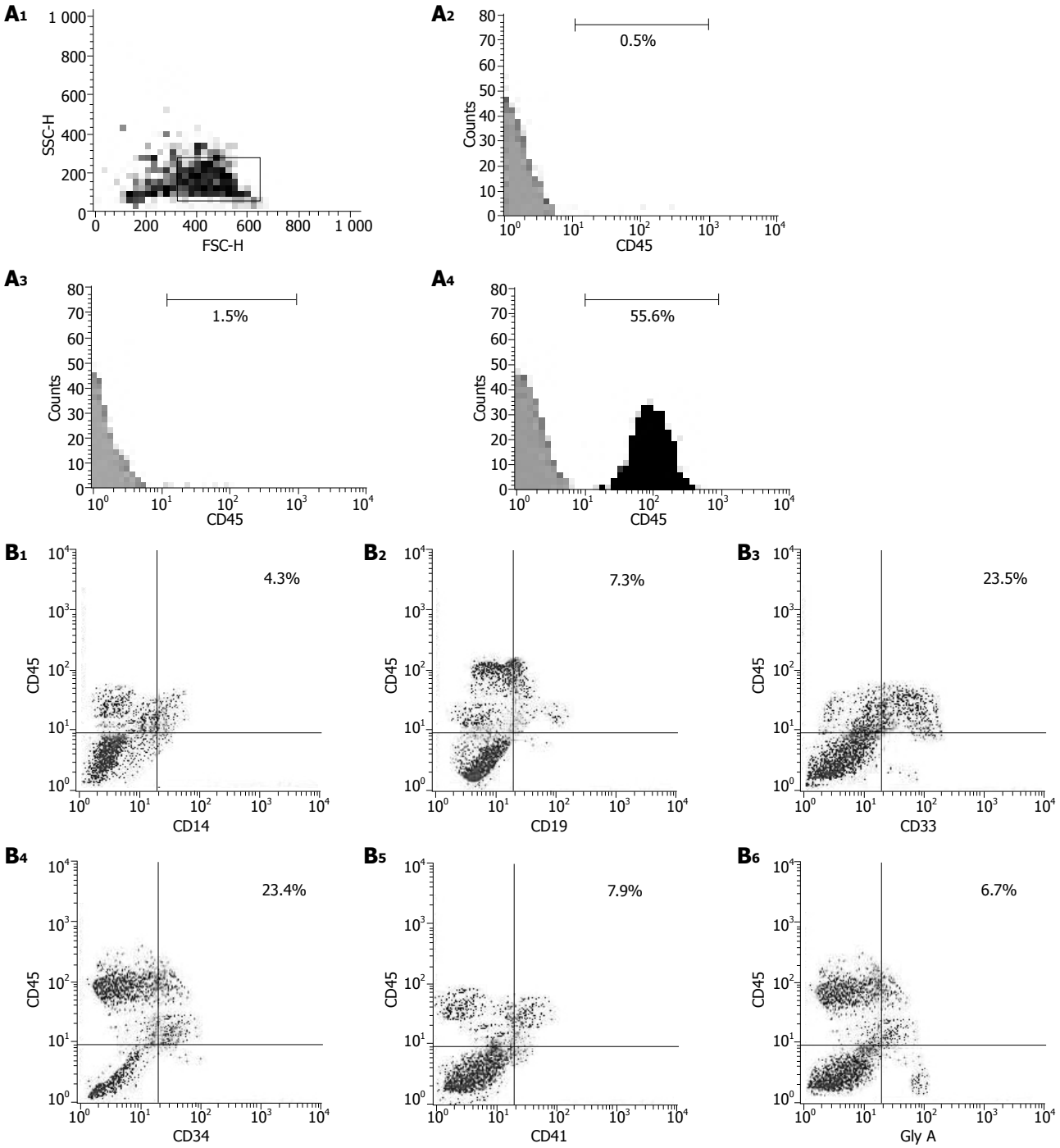

Figure 4 Determination of human hematopoietic reconstitution in NOD/SCID mice 7 wk after transplantation. **A:** Expression of human CD45 on bone marrow cells (BMC) collected from NOD/SCID mice 7 wk after transplantation; (**A1**): Cells in the gated area were analyzed by flow cytometry; (**A2**): Bone marrow cells (BMC) from an untransplanted mouse (negative control); (**A3**): BMC from a mouse transplanted with 1 ×10^5 human $CD34^+$ cells before culture; (**A4**): BMC from a mouse transplanted with cells obtained after 14-d cultivation of those 100 000 human $CD34^+$ cells in the tfhMSCs coculture system; **B1-B6:** Specific subsets of human $CD45^+$ cells in bone marrow cells of the NOD/SCID mouse transplanted with UCB cells after the coculture. Harvested cells were stained with PE-conjugated CD45 and various FITC-conjugated monoclonal antibodies.

expanded hematopoietic progenitors were expected to sustain long-term hematopoiesis. As a result, 10.23-fold LTC-IC amplification was observed in cells expanded by the tfhMSCs coculture system, although LTC-IC frequency was decreased during culture. The SRC assay indicated the reconstituting ability of these cultured human PPC. Although we could not perform a quantitative SRC assay, the difference in the percentage of chimerism of human $CD45^+$ cells between bone marrow cells of mice transplanted with cultured cells and those transplanted with control samples strongly suggested the extensive ability of these ex vivo-generated PPC to sustain and reconstitute long-term human hematopoiesis in vivo. Furthermore, PCR analysis confirmed flow cytometric results.

In conclusion, the main obstacle to UCB transplantation in adult recipients is the insufficiency of hematopoietic progenitors. A novel tfhMSCs coculture

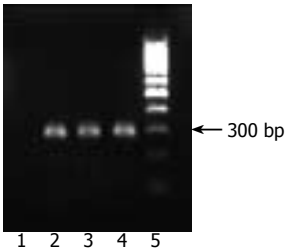

Figure 5 PCR analysis of human Alu sequence in NOD/SCID mice 7 wk after transplantation. DNA was extracted from the bone marrow and peripheral blood cells of NOD/SCID mice 7 wk after transplantation. PCR analysis demonstrated the presence of human hematopoietic cells in the bone marrow (lane 2) and peripheral blood cells (lane 3) of NOD/SCID mice. DNA extracted from human UCB cells was used as a positive control (lane 4). Negative control is shown in lane 1 and DNA marker is shown in lane 5.

system that could efficiently expand UCB hematopoietic progenitors is successfully established. The extent of LTC-IC expansion shown herein has important practical implications in terms of clinical hematopoietic stem cell transplantation.

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REFERENCES

1 **Osawa M**, Hanada K, Hamada H, Nakauchi H. Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* 1996; **273**: 242-245
2 **Wang JC**, Doedens M, Dick JE. Primitive human hematopoietic cells are enriched in cord blood compared with adult bone marrow or mobilized peripheral blood as measured by the quantitative in vivo SCID-repopulating cell assay. *Blood* 1997; **89**: 3919-3924
3 **Broxmeyer HE**, Douglas GW, Hangoc G, Cooper S, Bard J, English D, Arny M, Thomas L, Boyse EA. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. *Proc Natl Acad Sci U S A* 1989; **86**: 3828-3832
4 **Cairo MS**, Wagner JE. Placental and/or umbilical cord blood: an alternative source of hematopoietic stem cells for transplantation. *Blood* 1997; **90**: 4665-4678
5 **Kurtzberg J**, Laughlin M, Graham ML, Smith C, Olson JF, Halperin EC, Ciocci G, Carrier C, Stevens CE, Rubinstein P. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. *N Engl J Med* 1996; **335**: 157-166
6 **Petzer AL**, Zandstra PW, Piret JM, Eaves CJ. Differential cytokine effects on primitive (CD34+CD38-) human hematopoietic cells: novel responses to Flt3-ligand and thrombopoietin. *J Exp Med* 1996; **183**: 2551-2558
7 **McNiece I**, Kubegov D, Kerzic P, Shpall EJ, Gross S. Increased expansion and differentiation of cord blood products using a two-step expansion culture. *Exp Hematol* 2000; **28**: 1181-1186
8 **Conneally E**, Cashman J, Petzer A, Eaves C. Expansion in vitro of transplantable human cord blood stem cells demonstrated using a quantitative assay of their lympho-myeloid repopulating activity in nonobese diabetic-scid/scid mice. *Proc Natl Acad Sci U S A* 1997; **94**: 9836-9841
9 **Ueda T**, Tsuji K, Yoshino H, Ebihara Y, Yagasaki H, Hisakawa H, Mitsui T, Manabe A, Tanaka R, Kobayashi K, Ito M, Yasukawa K, Nakahata T. Expansion of human NOD/SCID-repopulating cells by stem cell factor, Flk2/Flt3 ligand, thrombopoietin, IL-6, and soluble IL-6 receptor. *J Clin Invest* 2000; **105**: 1013-1021
10 **Moore KA**, Ema H, Lemischka IR. In vitro maintenance of highly purified, transplantable hematopoietic stem cells. *Blood* 1997; **89**: 4337-4347
11 **Xu MJ**, Tsuji K, Ueda T, Mukouyama YS, Hara T, Yang FC, Ebihara Y, Matsuoka S, Manabe A, Kikuchi A, Ito M, Miyajima A, Nakahata T. Stimulation of mouse and human primitive hematopoiesis by murine embryonic aorta-gonad-mesonephros-derived stromal cell lines. *Blood* 1998; **92**: 2032-2040
12 **Kanai M**, Hirayama F, Yamaguchi M, Ohkawara J, Sato N, Fukazawa K, Yamashita K, Kuwabara M, Ikeda H, Ikebuchi K. Stromal cell-dependent ex vivo expansion of human cord blood progenitors and augmentation of transplantable stem cell activity. *Bone Marrow Transplant* 2000; **26**: 837-844
13 **Kusadasi N**, Koevoet JL, van Soest PL, Ploemacher RE. Stromal support augments extended long-term ex vivo expansion of hemopoietic progenitor cells. *Leukemia* 2001; **15**: 1347-1358
14 **Kawada H**, Ando K, Tsuji T, Shimakura Y, Nakamura Y, Chargui J, Hagihara M, Itagaki H, Shimizu T, Inokuchi S, Kato S, Hotta T. Rapid ex vivo expansion of human umbilical cord hematopoietic progenitors using a novel culture system. *Exp Hematol* 1999; **27**: 904-915
15 **Shimakura Y**, Kawada H, Ando K, Sato T, Nakamura Y, Tsuji T, Kato S, Hotta T. Murine stromal cell line HESS-5 maintains reconstituting ability of Ex vivo-generated hematopoietic stem cells from human bone marrow and cytokine-mobilized peripheral blood. *Stem Cells* 2000; **18**: 183-189
16 **Breems DA**, Blokland EA, Siebel KE, Mayen AE, Engels LJ, Ploemacher RE. Stroma-contact prevents loss of hematopoietic stem cell quality during ex vivo expansion of CD34+ mobilized peripheral blood stem cells. *Blood* 1998; **91**: 111-117
17 **Bennaceur-Griscelli A**, Tourino C, Izac B, Vainchenker W, Coulombel L. Murine stromal cells counteract the loss of long-term culture-initiating cell potential induced by cytokines in CD34(+)CD38(low/neg) human bone marrow cells. *Blood* 1999; **94**: 529-538
18 **Gan OI**, Murdoch B, Larochelle A, Dick JE. Differential maintenance of primitive human SCID-repopulating cells, clonogenic progenitors, and long-term culture-initiating cells after incubation on human bone marrow stromal cells. *Blood* 1997; **90**: 641-650
19 **Yamaguchi M**, Hirayama F, Kanai M, Sato N, Fukazawa K, Yamashita K, Sawada K, Koike T, Kuwabara M, Ikeda H, Ikebuchi K. Serum-free coculture system for ex vivo expansion of human cord blood primitive progenitors and SCID mouse-reconstituting cells using human bone marrow primary stromal cells. *Exp Hematol* 2001; **29**: 174-182
20 **Harley CB**, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature* 1990; **345**: 458-460
21 **Koli K**, Keski-Oja J. Cellular senescence. *Ann Med* 1992; **24**: 313-318
22 **Roecklein BA**, Torok-Storb B. Functionally distinct human marrow stromal cell lines immortalized by transduction with the human papilloma virus E6/E7 genes. *Blood* 1995; **85**: 997-1005
23 **Loeuillet C**, Bernard G, Rémy-Martin J, Saas P, Hervé P, Douay L, Chalmers D. Distinct hematopoietic support by two human stromal cell lines. *Exp Hematol* 2001; **29**: 736-745
24 **Garrido SM**, Appelbaum FR, Willman CL, Banker DE. Acute myeloid leukemia cells are protected from spontaneous and drug-induced apoptosis by direct contact with a human bone marrow stromal cell line (HS-5). *Exp Hematol* 2001; **29**: 448-457
25 **Novotny JR**, Duehrsen U, Welch K, Layton JE, Cebon JS, Boyd AW. Cloned stromal cell lines derived from human Whitlock/Witte-type long-term bone marrow cultures. *Exp Hematol* 1990; **18**: 775-784
26 **Zhang Y**, Li C, Jiang X, Zhang S, Wu Y, Liu B, Tang P, Mao N. Human placenta-derived mesenchymal progenitor cells support culture expansion of long-term culture-initiating cells from cord blood CD34+ cells. *Exp Hematol* 2004; **32**: 657-664

27 **Wang JF**, Wang LJ, Wu YF, Xiang Y, Xie CG, Jia BB, Harrington J, McNiece IK. Mesenchymal stem/progenitor cells in human umbilical cord blood as support for ex vivo expansion of CD34(+) hematopoietic stem cells and for chondrogenic differentiation. *Haematologica* 2004; **89**: 837-844

28 **Sutherland HJ**, Lansdorp PM, Henkelman DH, Eaves AC, Eaves CJ. Functional characterization of individual human hematopoietic stem cells cultured at limiting dilution on supportive marrow stromal layers. *Proc Natl Acad Sci U S A* 1990; **87**: 3584-3588

29 **Bhatia M**, Wang JC, Kapp U, Bonnet D, Dick JE. Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. *Proc Natl Acad Sci U S A* 1997; **94**: 5320-5325

30 **Haynesworth SE**, Baber MA, Caplan AI. Cytokine expression by human marrow-derived mesenchymal progenitor cells in vitro: effects of dexamethasone and IL-1 alpha. *J Cell Physiol* 1996; **166**: 585-592

31 **Majka M**, Janowska-Wieczorek A, Ratajczak J, Ehrenman K, Pietrzkowski Z, Kowalska MA, Gewirtz AM, Emerson SG, Ratajczak MZ. Numerous growth factors, cytokines, and chemokines are secreted by human CD34(+) cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner. *Blood* 2001; **97**: 3075-3085

32 **Piacibello W**, Sanavio F, Garetto L, Severino A, Bergandi D, Ferrario J, Fagioli F, Berger M, Aglietta M. Extensive amplification and self-renewal of human primitive hematopoietic stem cells from cord blood. *Blood* 1997; **89**: 2644-2653

33 **Kawano Y**, Kobune M, Yamaguchi M, Nakamura K, Ito Y, Sasaki K, Takahashi S, Nakamura T, Chiba H, Sato T, Matsunaga T, Azuma H, Ikebuchi K, Ikeda H, Kato J, Niitsu Y, Hamada H. Ex vivo expansion of human umbilical cord hematopoietic progenitor cells using a coculture system with human telomerase catalytic subunit (hTERT)-transfected human stromal cells. *Blood* 2003; **101**: 532-540

34 **Briddell RA**, Broudy VC, Bruno E, Brandt JE, Srour EF, Hoffman R. Further phenotypic characterization and isolation of human hematopoietic progenitor cells using a monoclonal antibody to the c-kit receptor. *Blood* 1992; **79**: 3159-3167

35 **Traycoff CM**, Abboud MR, Laver J, Brandt JE, Hoffman R, Law P, Ishizawa L, Srour EF. Evaluation of the in vitro behavior of phenotypically defined populations of umbilical cord blood hematopoietic progenitor cells. *Exp Hematol* 1994; **22**: 215-222

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BASIC RESEARCH

Role of Kupffer cells in acute hemorrhagic necrotizing pancreatitis-associated lung injury of rats

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Abstract

AIM: To investigate the role of Kupffer cells (KCs) in acute hemorrhagic necrotizing pancreatitis-associated lung injury (AHNP-LI).

METHODS: Forty-two rats were allocated to four groups [sham operation, AHNP model, gadolinium chloride ($GdCl_3$) pretreatment, $GdCl_3$ control]. In $GdCl_3$ pretreatment group, $GdCl_3$ was administered by caudal vein injection 24 h before the AHNP model induction. Blood from the iliac artery, alveolar macrophages and tissues from the pancreas and lung, were collected in six animals per group 3 and 6 h after acute pancreatitis induction. TNF-α, IL-1 of serum, myeloperoxidase (MPO) of lung tissue, NF-κB activation of alveolar macrophages were detected. Serum AST and ALT in sham operation group and $GdCl_3$ control group were tested. In addition, histopathological changes of the pancreas and lung were observed under light microscope.

RESULTS: MPO of lung tissue and TNF-α, IL-1 levels of serum were all reduced significantly in $GdCl_3$ pretreatment group compared to those in AHNP group ($P<0.01$). NF-κB activation of alveolar macrophages was also attenuated significantly in $GdCl_3$ pretreatment group compared to that in AHNP group ($P<0.01$). The pathological injury of the lung was ameliorated obviously in $GdCl_3$ pretreatment group compared to that in AHNP group. Nevertheless, the serum amylase level did not reduce and injury of the pancreas was not prevented in $GdCl_3$ pretreatment group.

CONCLUSION: Pulmonary injury induced by AHNP is mediated by KC activation and AHNP-LI can be significantly ameliorated by pretreatment with $GdCl_3$ and KCs play a vital role in AHNP-LI.

Key words: Pancreatitis-associated lung injury; Kupffer cell; NF-κB; Gadolinium chloride

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INTRODUCTION

Acute lung injury is a severe complication of acute hemorrhagic necrotizing pancreatitis (AHNP)[1-3]. However, its pathogenic mechanism is not well understood and its treatment remains supportive. Recent researches suggest that inflammatory cytokines derived from the liver, especially hepatic cytokine released from Kupffer cells (KCs) may cause distant organ failure and death in severe pancreatitis and that KCs are an important source of inflammatory cytokines and may be the main factor causing lung damage in AHNP[4,5]. Here we studied the KC contribution to lung injury associated with AHNP. The present study was to investigate the role of KCs in acute hemorrhagic necrotizing pancreatitis-associated lung injury.

MATERIALS AND METHODS

Experimental animals, agents, and ELISA kits

Male Wistar rats were provided by Experimental Animal Center of Capital Medical University (Beijing). Sodium taurocholate and gadolinium chloride ($GdCl_3$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). TNF-α, IL-1 ELISA kits were purchased from TPI Ltd. (USA). TransAM™ NF-κB P^{65} chemi ELISA kit was provided by Active Motif (USA). Central LB 960 microplate luminometer was from Berthold Ltd.

Induction of AHNP model

Experimental AHNP was induced as previously described[6]. Briefly, a small median laparotomy was performed, then the pancreas was exteriorized and the hepatic duct was closed at the liver hilum with a soft microvascular clamp to prevent reflux of the infused

material into the liver. The biliopancreatic duct was cannulated through the duodenum and 5% sodium taurocholate (1 mL/kg body weight, 0.1 mL/min) was retrogradely injected into the biliopancreatic duct. The clamp was removed 5 min after the injection. The abdominal wound was closed, and the animals were sent back to their cages with free access to water and food after surgery.

Animals

Forty-two male Wistar rats (weighing 250-280 g) were randomly divided into four groups: sham operation, AHNP model, $GdCl_3$ pretreatment and $GdCl_3$ control. In the $GdCl_3$ pretreatment group, $GdCl_3$ solution (4%, 10 mg/kg) was administrated by caudal vein injection 24 h before the AHNP model was established. Blood from the iliac artery, alveolar macrophages, and tissues from the pancreas and lung, were collected in six animals per group 3 and 6 h after acute pancreatitis induction. Serum levels of TNF-α and IL-1 were determined by enzyme-linked immunosorbent assay. Myeloperoxidase (MPO) level in the lung and NF-κB activation of the alveolar macrophages were detected. Serum AST and ALT in sham operation group and $GdCl_3$ control group were tested by biochemical method. In addition, histopathological changes of the pancreas and lung were observed under light microscope.

Alveolar macrophage isolation and nuclear protein extraction

Bronchoalveolar lavage (BAL) was performed five times in the left lung, using 5 mL of sterile normal saline per lavage given through a tracheal cannula[7]. The whole BAL fluid (BALF) was centrifuged at 280 r/min for 10 min at 4 ℃. Cell pellets were resuspended (1×10^5 cells/mL) in RPMI 1640 medium. Cell suspension was then placed in plastic petri dishes (Nunc, Denmark) and incubated at 37 ℃ for 1 h in a CO_2 incubator (50 mL/L CO_2+95% air). Non-adherent cells were removed from adherent macrophages by washing with RPMI 1640 medium. Purified alveolar macrophages were recovered by gently rubbing the dishes with a rubber policeman. Nuclear protein was extracted from purified alveolar macrophages as previously described[8]. Protein content was determined by Bradford method, stored at -70 ℃ for subsequent examination of NF-κB activity.

Amylase estimation

Serum amylase activity was measured by iodoamylum method and expressed as U/L.

Serum TNF-α, IL-1 estimation

Serum TNF-α and IL-1 were measured by ELISA method according to the instructions of the kits.

Lung tissue MPO estimation

Lung tissue MPO activity was detected according to the instructions of commercial kit.

Serum AST and ALT estimation

Serum levels of AST and ALT were determined using Toshiba VF-A5/A5P Bio-Chemical analyzer.

Table 1 Serum amylase in all experimental groups (U/L, mean±SD)

Group	Serum amylase	
	3 h ($n=6$)	6 h ($n=6$)
Sham	499.4±86.3	503.8±91.2
AHNP	10 444.5±1 863.2[b]	13 316.4±1 374.1[b]
$GdCl_3$ pretreatment	9 107.1±569.9[b]	12 420.4±1 779.2[b]

[b]$P<0.001$ *vs* sham group.

Table 2 Influence of $GdCl_3$ on hepatic functions of experimental animals (U/L, mean±SD)

Group	*n*	Serum AST	Serum ALT
Sham 3 h	6	74.2±13.6	31.6±9.3
Sham 6 h	6	71.3±15.0	32.7±11.3
$GdCl_3$ control	6	82.5±14.6	28.9±8.7

NF-κB of alveolar macrophage estimation

NF-κB activation of alveolar macrophages was determined using TransAM™ NF-κB P^{65} chemi ELISA kit by Central LB 960 microplate luminometer and expressed as relative light units (RLU).

Histology

Pancreas and lung tissues were collected and evaluated by a pathologist blinded to the experimental assignment of the animals. After embedded with paraffin, the tissue was stained with hematoxylin-eosin (H&E). Pulmonary lesion was scored following Lei's criteria[9].

Statistical analysis

All data were expressed as mean±SD. Comparisons among multiple experimental groups and between each time point were made using ANOVA. $P<0.05$ was considered statistically significant.

RESULTS

Table 1 illustrates the levels of serum amylase in all the three groups. Three and six hours after AHNP induction, serum amylase increased significantly in AHNP group compared to that in sham operation group. But there was no significant difference between $GdCl_3$ pretreatment group and AHNP group 3 and 6 h after AHNP induction.

Table 2 shows the influence of $GdCl_3$ on hepatic functions of experimental animals. No changes were found in $GdCl_3$-treated animals and the values of ALT and AST in $GdCl_3$ control group were similar to those in sham group measured at all time points.

Serum TNF-α and IL-1 in AHNP group were significantly increased compared to those measured in sham group at all time points ($P<0.01$). In $GdCl_3$ pretreatment group, 3 and 6 h after AHNP induction, serum TNF-α and IL-1 were significantly decreased ($P<0.01$, Table 3).

Table 4 shows the MPO activity of lung injury in

Table 3 Serum TNF-α and IL-1 in all experimental groups (mean±SD)

Group	*n*	TNF-α (pg/mL)	IL-1 (μg/L)
3 h			
Sham	6	32.0±8.6	0.29±0.08
AHNP	6	113.9±18.5	0.81±0.11
$GdCl_3$ pretreatment	6	71.1±14.3[b]	0.49±0.12[b]
6 h			
Sham	6	31.9±8.2	0.28±0.07
AHNP	6	126.3±12.6	0.91±0.12
$GdCl_3$ pretreatment	6	78.0±17.2[b]	0.53±0.13[b]

[b]$P<0.01$ *vs* AHNP group.

Table 4 Lung tissue MPO level in all groups (mU/mg prot, mean±SD)

Group	MPO	
	3 h (*n* = 6)	6 h (*n* = 6)
Sham	0.51±0.13	0.50±0.17
AHNP	1.59±0.26	1.79±0.23
$GdCl_3$ pretreatment	0.94±0.15[b]	1.02±0.19[b]

[b]$P<0.01$ *vs* AHNP group.

Table 5 Histopathologic scores of lung tissue in all groups (mean±SD)

Group	Lung histopathologic scores	
	3 h (*n* = 6)	6 h (*n* = 6)
Sham	0.17±0.04	0.17±0.04
AHNP	2.50±0.55[b]	2.67±0.52[b]
$GdCl_3$ pretreatment	1.87±0.41[a]	2.00±0.38[a]

[a]$P<0.05$ *vs* AHNP group; [b]$P<0.01$ *vs* sham operation group.

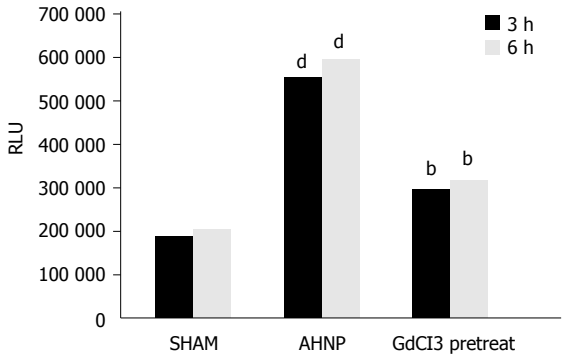

Figure 1 NF-κB activity of alveolar macrophages in BALF of all groups. [b]$P<0.01$ *vs* AHNP group, [d]$P<0.01$ *vs* sham operation group.

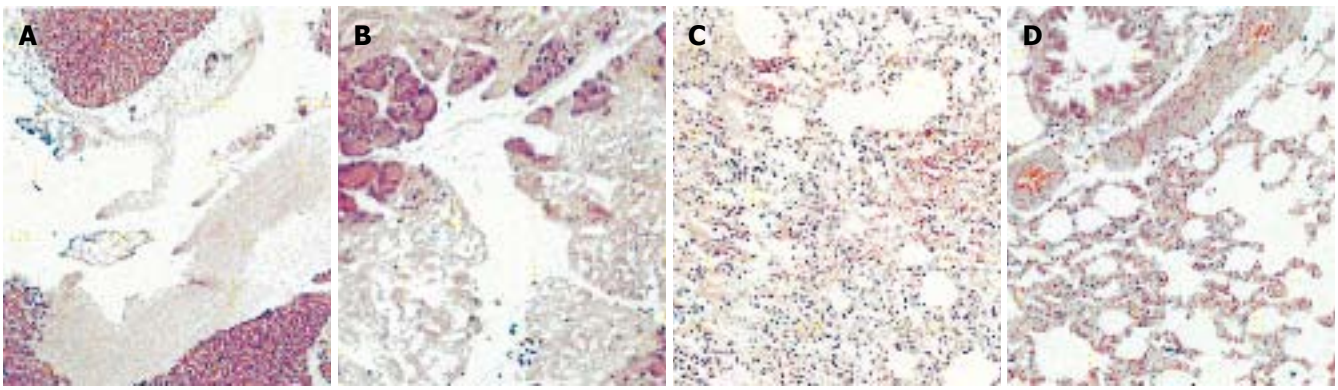

Figure 2 Histopathological changes in different groups. **A**: Extensive necrosis, intense edema and inflammatory infiltrate in AHNP group (100×); **B**: extensive necrosis and intense edema in $GdCl_3$ pretreatment group (100×); **C**: diffuse alveolar blood stasis and heavy infiltration of inflammatory cells (100×) in AHNP group; **D**: mild edema of the alveolar walls and mild alveolar blood stasis with slight infiltration of neutrophils in $GdCl_3$ pretreatment group (100×).

all the groups. The MPO levels in AHNP group were significantly higher than those in sham operation group ($P<0.01$). In $GdCl_3$ pretreatment group, 3 and 6 h after AHNP induction, the lung MPO levels were significantly decreased ($P<0.01$).

Figure 1 shows that the NF-κB activity of alveolar macrophages in AHNP group was significantly higher than that in sham operation group ($P<0.01$) and was significantly decreased in $GdCl_3$ pretreatment group ($P<0.01$).

Histopathological study of the pancreas, 3 and 6 h after AHNP induction (Figure 2A) revealed extensive necrosis of pancreatic tissue, intense edema, and inflammatory infiltrate. The necrosis of pancreatic tissue and edema in $GdCl_3$ pretreatment group were similar to those in AHNP group (Figure 2B). The sham operation group was normal.

Diffuse alveolar blood stasis, intense alveolar septum swelling and heavy infiltration of inflammatory cells mostly neutrophils were found in the lung tissue of AHNP group (Figure 2C). The mean histopathologic scores of AHNP group were significantly higher than those in sham operation group ($P<0.01$, Table 5). In $GdCl_3$ pretreatment group, the major histopathological findings were mild edema of the alveolar walls and mild alveolar blood stasis with slight infiltration of neutrophils (Figure 2D). The mean histopathologic scores of $GdCl_3$ pretreatment group decreased significantly compared to those of AHNP group ($P<0.05$).

DISCUSSION

Acute hemorrhagic necrotizing pancreatitis (AHNP) is a potentially fatal disease with a morbidity and mortality rate of approximately 30%. Acute lung injury (ALI)

is a common complication of AHNP, but the events that link AHNP and pulmonary damage are not fully understood. Many factors, such as oxygen free radicals, platelet activating factor, phospholipase A_2 (PLA_2), cyclooxygenase-2 (COX-2), cytokines and arachidonic acid metabolites are related to AHNP and ALI[10-13]. Pancreatic proteolytic enzymes or activated PLA_2 released into the circulatory system determines the development of lung injury[14]. Furthermore, other mediators in lung tissue such as platelet activating factor, arachidonic acid metabolites can stimulate inflammatory cell activation[15,16]. Also, interaction of polymorphonuclear granulocytes, endothelium, and endothelium-derived mediators seems to be important to amplify lung damage[17]. Recently, Cheng *et al*[18,19] noted that activation of alveolar macrophages may play an important role in lung injury associated with AHNP, and that TNF-α and nitric oxide (NO) secreted by alveolar macrophages are increased significantly in rats with AHNP. Bhatia *et al*[20] reported that inhibition of the production of hydrogen sulfide (H_2S) can significantly reduce the severity of cerulein-induced pancreatitis and associated-lung injury, suggesting an important proinflammatory role in regulating the severity of pancreatitis and associated-lung injury.

In recent years, some researchers found that the liver, especially KCs, might play a vital role in ALI caused by other different factors. Okutan *et al*[21] reported that KCs blocked by $GdCl_3$ can attenuate lung damage caused by aortic ischemia reperfusion and malondialdehyde (MDA) level, an indicator of free radical generation and MPO activity, an indirect evidence of neutrophil infiltration in lung injury are decreased significantly[21]. Although there is no evidence that $GdCl_3$ can suppress the function of neutrophils, it was reported that $GdCl_3$ can suppress the accumulation of neutrophils and alveolar macrophages[22]. Feng *et al*[23] investigated the role of KCs in the pathogenesis of ALI during acute obstructive cholangitis (AOC) and found that the phagocytic function of KCs is damaged in ALI induced by AOC.

KCs, the resident macrophages in the liver, are the major component of mononuclear phagocytic system (MPS). These macrophages make up 90% of the MPS and have abundant cytoplasma where abundant ribosome and phagosomes are located. These typical structures are associated with their functions. It was reported that KCs are responsible for the increased levels of TNF, IL-1, IL-6 in trauma, hemorrhagic shock and resuscitation. Decreasing the number or functional ability of KCs can lead to decreased levels of inflammatory cytokines as seen in the models of liver resection and sepsis[24,25].

Also, KCs are regarded as the predominant source of inflammatory cytokines in AHNP at present[26,27]. Closa *et al*[4,5] performed an end-to-side portacaval shunt before AHNP induction in rats and found that portacaval shunting appears to exert a profound effect on ameliorating the inflammatory infiltrate. It is suggested that almost all the pancreatic enzymes and mediators released from the pancreas into the plasma during AHNP pass through the liver before their dilution in the systemic circulation, indicating that this step is a determinant in the development of the lung injury response. These observations point to the key role of liver as a triggering mechanism for inflammatory processes in the lung as a consequence of AHNP. Moreover, activation of hepatic inflammatory cells, especially KCs, plays a key role in the development of lung injury[4,5].

To improve our understanding of the role of KCs in AHNP-ALI, $GdCl_3$ was given 24 h prior to AHNP model induction to eliminate KCs in the present study. Moreover, the dose of $GdCl_3$ used in our study was 10 mg/Kg because administration of $GdCl_3$ at a dose 10 mg/kg can block the phagocytic activity of KCs completely[28]. In $GdCl_3$ pretreatment group, the levels of MPO in lung tissue and the levels of TNF-α, IL-1 in serum all decreased significantly compared to those in AHNP group. NF-κB activation of alveolar macrophages was also attenuated significantly in $GdCl_3$ pretreatment group compared to that in AHNP group. All these data suggest that $GdCl_3$ can ameliorate AHNP-ALI significantly and these results are in accordance with those reported by Folch *et al*[29]. It was reported that lung injury associated with acute necrotic pancreatitis (ANP) is ameliorated by $GdCl_3$ through inducing apoptosis of alveolar macrophages of ANP[30].

In our study, the serum amylase level did not decrease and the injury of pancreas was not prevented in $GdCl_3$ pretreatment group, suggesting that $GdCl_3$ cannot prevent lung injury by ameliorating pancreatic injury.

Using $GdCl_3$ as a tool to investigate the role of KCs is more reasonable than portacaval shunting operation which appears to exert a profound effect on animal's systemic circulation. Serum AST and ALT estimation suggests that $GdCl_3$ has no harmful effects on hepatic functions.

In conclusion, KCs play a vital role in AHNP and ALI.

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REFERENCES

1 **Zhao X**, Andersson R, Wang X, Dib M, Wang X. Acute pancreatitis-associated lung injury: pathophysiological mechanisms and potential future therapies. *Scand J Gastroenterol* 2002; **37**: 1351-1358

2 **Liu YG**, Pan JJ, Niu LC, Yan QM, Song B, Li Y, Zhang WF. 18 cases report of severe acute pancreatitis-associated lung injury. *Zhongguo Putong Waike Zazhi* 2002; **11**: 182-184

3 **Wang Y**, Tian YX, Tan XD. The therapeutic methods of severe acute pancreatitis (94 cases report). *Zhongguo Putong Waike Zazhi* 2001; **10**: 302-304

4 **Closa D**, Sabater L, Fernández-Cruz L, Prats N, Gelpí E, Roselló-Catafau J. Activation of alveolar macrophages in lung injury associated with experimental acute pancreatitis is mediated by the liver. *Ann Surg* 1999; **229**: 230-236

5 **Closa D**, Bardají M, Hotter G, Prats N, Gelpí E, Fernández-Cruz L, Roselló-Catafau J. Hepatic involvement in pancreatitis-induced lung damage. *Am J Physiol* 1996; **270**: G6-13

6 **Aho HJ**, Koskensalo SM, Nevalainen TJ. Experimental pancreatitis in the rat. Sodium taurocholate-induced acute haemorrhagic pancreatitis. *Scand J Gastroenterol* 1980; **15**: 411-416

7 **Satoh A**, Shimosegawa T, Fujita M, Kimura K, Masamune A, Koizumi M, Toyota T. Inhibition of nuclear factor-kappaB activation improves the survival of rats with taurocholate pancreatitis. *Gut* 1999; **44**: 253-258

8 **Schreiber E**, Matthias P, Müller MM, Schaffner W. Rapid detection of octamer binding proteins with 'mini-extracts',

prepared from a small number of cells. *Nucleic Acids Res* 1989; **17**: 6419
9 **Lei WZ**, Wei JJ, Shen WL, Jin LR. The relationship between MODS of experimental necrotizing pancreatitis and endotoxinmia. *Zhongguo Shiyan Waike Zazhi* 1995; **12**: 131-133.
10 **Hardman J**, Shields C, Schofield D, McMahon R, Redmond HP, Siriwardena AK. Intravenous antioxidant modulation of end-organ damage in L-arginine-induced experimental acute pancreatitis. *Pancreatology* 2005; **5**: 380-386
11 **Takala A**, Jousela I, Takkunen O, Kautiainen H, Jansson SE, Orpana A, Karonen SL, Repo H. A prospective study of inflammation markers in patients at risk of indirect acute lung injury. *Shock* 2002; **17**: 252-257
12 **Ethridge RT**, Chung DH, Slogoff M, Ehlers RA, Hellmich MR, Rajaraman S, Saito H, Uchida T, Evers BM. Cyclooxygenase-2 gene disruption attenuates the severity of acute pancreatitis and pancreatitis-associated lung injury. *Gastroenterology* 2002; **123**: 1311-1322
13 **Song AM**, Bhagat L, Singh VP, Van Acker GG, Steer ML, Saluja AK. Inhibition of cyclooxygenase-2 ameliorates the severity of pancreatitis and associated lung injury. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G1166-G1174
14 **Edelson JD**, Vadas P, Villar J, Mullen JB, Pruzanski W. Acute lung injury induced by phospholipase A2. Structural and functional changes. *Am Rev Respir Dis* 1991; **143**: 1102-1109
15 **St John RC**, Dorinsky PM. Immunologic therapy for ARDS, septic shock, and multiple-organ failure. *Chest* 1993; **103**: 932-943
16 **Zhou W**, Chao W, Levine BA, Olson MS. Role of platelet-activating factor in hepatic responses after bile duct ligation in rats. *Am J Physiol* 1992; **263**: G587-G592
17 **Willemer S**, Feddersen CO, Karges W, Adler G. Lung injury in acute experimental pancreatitis in rats. I. Morphological studies. *Int J Pancreatol* 1991; **8**: 305-321
18 **Cheng S**, He S, Zhang J. [The role of alveolar macrophage activation in rats with lung injury associated with acute necrotizing pancreatitis]. *Zhonghua Waike Zazhi* 2002; **40**: 609-612
19 **Cheng S**, Zhao J, He SG, Song MM, Li ZH, Zhang YW. [The role of nitric oxide in lung injury associated with acute necrotizing pancreatitis]. *Zhonghua Wai Ke Za Zhi* 2003; **41**: 336-339
20 **Bhatia M**, Wong FL, Fu D, Lau HY, Moochhala SM, Moore PK. Role of hydrogen sulfide in acute pancreatitis and associated lung injury. *FASEB J* 2005; **19**: 623-625
21 **Okutan H**, Savas C, Ozguner IF, Yonden Z, Eren VC, Delibas N. Lung injury after aortic occlusion-reperfusion in rats: the role of gadolinium chloride. *Tohoku J Exp Med* 2004; **203**: 267-273
22 **Sato K**, Kadiiska MB, Ghio AJ, Corbett J, Fann YC, Holland SM, Thurman RG, Mason RP. In vivo lipid-derived free radical formation by NADPH oxidase in acute lung injury induced by lipopolysaccharide: a model for ARDS. *FASEB J* 2002; **16**: 1713-1720
23 **Feng HY**, Shi YJ, Wu CX, Li SW, Liu CA, Gong JP. Mononuclear macrophages in pathogenesis of acute lung injury during acute obstructive cholangitis. *Hepatobiliary Pancreat Dis Int* 2002; **1**: 587-591
24 **Savas C**, Ozogul C, Karaöz E, Delibas N, Ozgüner F. Splenectomy reduces remote organ damage after intestinal ischaemia-reperfusion injury. *Acta Chir Belg* 2003; **103**: 315-320
25 **Towfigh S**, Heisler T, Rigberg DA, Hines OJ, Chu J, McFadden DW, Chandler C. Intestinal ischemia and the gut-liver axis: an in vitro model. *J Surg Res* 2000; **88**: 160-164
26 **Gloor B**, Blinman TA, Rigberg DA, Todd KE, Lane JS, Hines OJ, Reber HA. Kupffer cell blockade reduces hepatic and systemic cytokine levels and lung injury in hemorrhagic pancreatitis in rats. *Pancreas* 2000; **21**: 414-420
27 **Jankov RP**, Luo X, Belcastro R, Copland I, Frndova H, Lye SJ, Hoidal JR, Post M, Tanswell AK. Gadolinium chloride inhibits pulmonary macrophage influx and prevents O(2)-induced pulmonary hypertension in the neonatal rat. *Pediatr Res* 2001; **50**: 172-183
28 **Tullis MJ**, Brown S, Gewertz BL. Hepatic influence on pulmonary neutrophil sequestration following intestinal ischemia-reperfusion. *J Surg Res* 1996; **66**: 143-146
29 **Folch E**, Prats N, Hotter G, López S, Gelpi E, Roselló-Catafau J, Closa D. P-selectin expression and Kupffer cell activation in rat acute pancreatitis. *Dig Dis Sci* 2000; **45**: 1535-1544
30 **Cheng S**, Song MM, Li ZH, He SG. [The protective role of gadolinium chloride in lung injury associated with acute necrotizing pancreatitis]. *Zhonghua Waike Zazhi* 2004; **42**: 936-939

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BASIC RESEARCH

Portal vein embolization induces compensatory hypertrophy of remnant liver

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Abstract

AIM: To evaluate the effectiveness and safety of different portal vein branch embolization agents in inducing compensatory hypertrophy of the remnant liver and to offer a theoretic basis for clinical portal vein branch embolization.

METHODS: Forty-one adult dogs were included in the experiment and divided into four groups. Five dogs served as a control group, 12 as a gelfoam group, 12 as a coil-gelfoam group and 12 as an absolute ethanol group. Left portal vein embolization was performed in each group. The results from the embolization in each group using different embolic agents were compared. The safety of portal vein embolization (PVE) was evaluated by liver function test, computed tomography (CT) and digital subtraction angiography (DSA) of liver and portal veins. Statistical test of variance was performed to analyze the results.

RESULTS: Gelfoam used for PVE was inefficient in recanalization of portal vein branch 4 wk after the procedure. The liver volume in groups of coil-gelfoam and absolute ethanol increased 25.1% and 33.18%, respectively. There was no evidence of recanalization of embolized portal vein, hepatic dysfunction, and portal hypertension in coil-gelfoam group and absolute ethanol group.

CONCOUSION: Portal vein branch embolization using absolute ethanol and coil-gelfoam could induce atrophy of the embolized lobes and compensatory hypertrophy of the remnant liver. Gelfoam is an inefficient agent.

Key words: Portal vein embolization; Interventional therapy; Liver; Dog

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INTRODUCTION

Primary carcinoma of the liver is one of the common malignant tumors in our country. Hepatectomy remains one of the most important therapies. However, only 15-30% of diagnosed patients are suitable for hepatectomy. Although extended hepatic resection is an effective therapy, partial excision of normal and functional liver is unavoidable in this surgery. If partial excision resects a certain amount of liver, patients especially those with liver cirrhosis, would suffer from postoperative liver failure. Although limiting resection area can reduce the possibility of complications, it increases the incidence of postoperative recurrence and metastasis. These problems can be effectively solved by artificially inducing hyperplasia of normal liver tissue preoperatively. Therefore, preoperative portal vein embolization (PPVE) is an effective procedure to settle this contradiction[1-5].

In 1920, Roust Larimore discovered that ligation of rabbits' portal vein branch could contribute to the atrophy of pathological lobe and hyperplasia of non-pathological one. In 1975, Honjo *et al*[6] tried portal vein ligation in liver cancer patients not fit for hepatic resection, which resulted in atrophy of the ligated lobe of portal vein and its tumor and hyperplasia of non ligated lobe. In 1980s, Makuuchi *et al*[7] found that if the portal vein of one lobe is embolized by tumor thrombi, hyperplasia usually occurs in the other lobe of the liver, and meanwhile, patients enjoy better recovery after hepatectomy because unexpected rising of portal vein pressure could be avoided. These findings have led to the exploration of preoperative PVE prior to extensive liver hepatic resection. PVE in clinical practice[1,2,8,9] and experimental studies[10-13] has achieved success. Since foreign emboli materials are expensive and difficult to obtain, it is essential to screen for easily-obtained, convenient, cheap, and safe embolic agents to effectively induce compensatory hyperplasia. The purpose of our study was to evaluate the effectiveness and safety of three convenient and cheap agents in inducing compensatory hypertrophy of liver.

MATERIALS AND METHODS

Experimental animals

Forty-one healthy adult dogs weighing 16.2+4.2 kg (range, 12.0-20.0 kg) without obvious growth (to avoid sudden changes of weight during the process of experiment, which would affect liver volume and influence experimental results), were included in the study. All dogs were provided by Experimental Animal Center of Union Hospital attached to Fujian Medical University. Before and after the experiment, they were fed with same standard forage. Five served as the control group and the remaining 36 were divided into 3 groups at random, 12 in each group. Gelfoam, coil-gelfoam and absolute ethanol were applied to these 3 experimental groups for portal vein embolization.

Methods

A dog with jejunitis on operative day was given an intravenous injection of 10% pentobarbital sodium into the hind legs to induce general anesthesia. Then, the dog underwent helical CT scanning to conduct pre and post contrast-enhanced CT study of its liver. The liver volume was measured automatically by a computer with the help of SOMATOM PLUS4 spiral CT scanner (Siemens Co.). The dog was transferred into the interventional operating room and cut open for catheter placement of portal vein under general anesthesia. After a 5F Cobra catheter was inserted into the trunk of portal vein, 76% angiografin was injected at the speed of 8-10 mL/s through the catheter to study the branch pattern of portal vein. Because the right lobe was relatively fixed, left portal vein was chosen as a target vessel for embolization. Then, the catheter was inserted into the left portal vein and saline or an embolic agent was injected into the appropriate position in different experimental groups. Control group: 30-50 mL saline was injected through the catheter. Gelfoam group: gelfoam was cut into strips of 10 mm×1.0 mm, which were inserted into the embolized portal vein through the catheter with saline until complete embolization was achieved. Embolization procedure was stopped when the embolized portal vein was completely embolized and the distal portal vein branch was not opacified. The number of gelfoams used ranged between 40-60 strips. Coil-gelfoam group: gelfoam was used to embolize smaller branches of the left portal vein, then coils were used to embolize the trunk of the left portal vein until satisfactory results were achieved. The number of coils ranged between 3-5. Absolute ethanol group: Five milliliters of absolute ethanol was slowly injected through the catheter. After several minutes, an additional amount of absolute ethanol was provided based on the embolization situation of portal vein in order to achieve the result of complete embolization. The amount of absolute ethanol was generally 0.5-1.0 mL/kg. After embolization, 9-12 mL angiografin was injected through the catheter at a speed of 6-8 mL/s to carry out angiography of the portal vein, which would allow us to understand its iconography expression after embolization. At the same time, we measured the pressure of main portal vein, and the radial lines of the nonembolized portal veins including the right portal vein and main portal vein.

Table 1 Changes of the right lobe volume in gelfoam group (cm^3)

No	Pre-embolization	4W	6W	8W
Dog A1	250	265	260	263
Dog A2	338	340	334	348
Dog A3	380	395	370	398
Dog A4	273	279	277	280
Dog A5	370	368	373	378
Dog A6	320	318	334	332
Dog A7	229	245	230	237
Dog A8	342	365	344	377
Dog A9	360	353	373	374
Dog A10	362	363	370	368
Mean±SD	318±18.2	347.6±21.4	321.7±17.7	331.9±19.4

After embolization, all dogs were regularly fed. Angiography was performed 4 and 8 wk after embolization, transcatheter portal vein angiography was undertaken to understand the iconography expression of portal vein branches, and then diameter of the nonembolized portal vein was measured. Changes of portal vein pressure were monitored 4 and 8 wk after embolization, transcatheter portal vein angiography was performed to measure the portal vein pressure. Changes of liver function were detected before and after embolization, alanine aminotransferase (ALT) was assayed every 2 d until liver function became normal. Changes of liver volume were determined 4, 6, and 8 wk after embolization, liver CT scanning was performed to measure the volume of the nonembolized lobe. Dogs were killed 8 wk after embolization, pathology observation was implemented of liver tissues of the embolized and nonembolized lobes.

Statistical analysis

SPSS11.0 software package was adopted to carry out statistical analysis of experimental results.

RESULTS

Control group

During the period of embolization, all the five dogs survived. Iconography expression was the same as before, suggesting that no blood vessel was embolized. Meanwhile, there were no changes of portal vein pressure, volume of the right lobe, liver function, and pathology before and after saline injection.

Gelfoam group

One dog died of respiratory failure, another dog died of acute liver failure due to gelfoam back-flow which led to extensive embolization of portal vein branches in liver, and 10 dogs survived. Post embolization angiography showed that the left branch of portal vein was completely embolized, while the right branch was not obstructed, the right portal vein and main portal vein were dilated compared to pre-embolization. Four weeks after embolization the embolized left portal vein had blood flow (Figure 1). The portal vein pressure was 94.1±1.8 mm H_2O, 123.2±2.8 mm H_2O before embolization, and 94.7±2.0 mm H_2O, 95.8±6.6 mm H_2O after embolization, respectively (P>0.05). ALT value obviously increased 2 d after embolization (P<0.01), but decreased subsequently

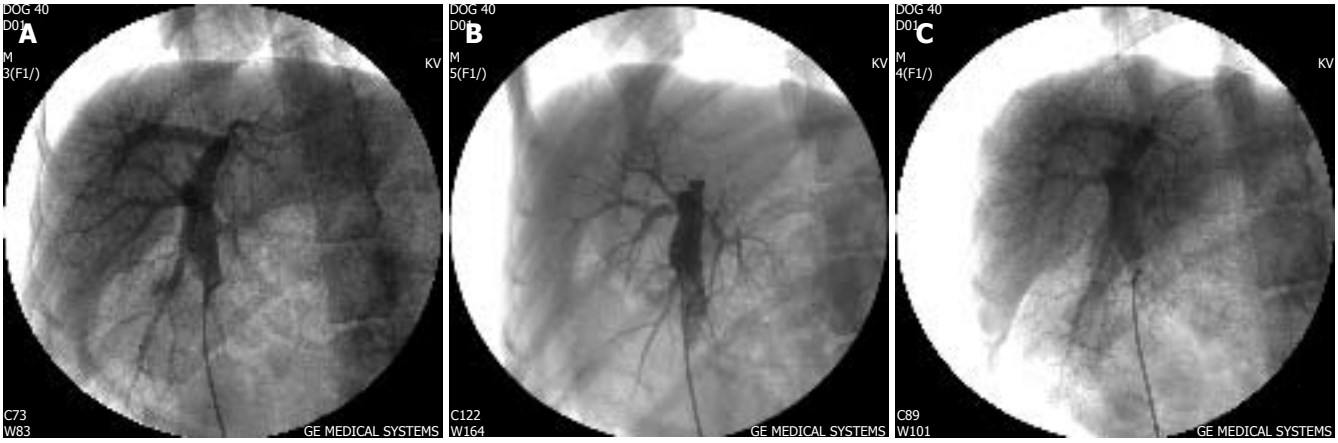

Figure 1 Portal vein angiography before (**A**) and after (**B**) embolization as well as 4 wk (**C**) after embolization with gelfoam.

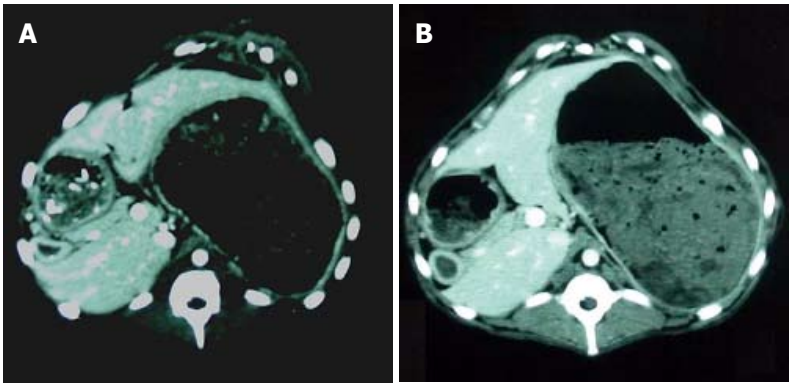

Figure 2 No obvious changes in volume of the right lobe before (**A**) and after (**B**) embolization.

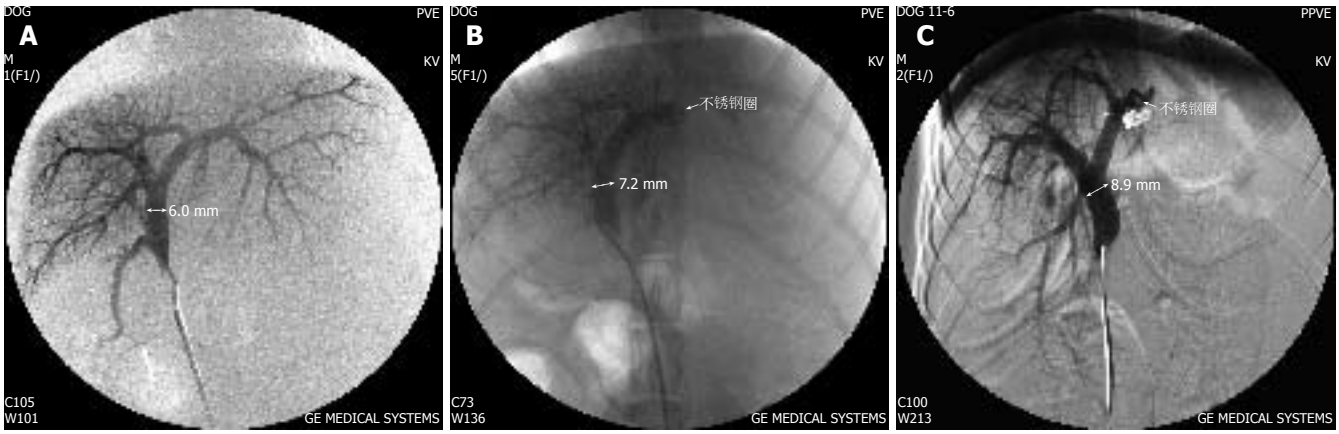

Figure 3 Portal vein angiography before (**A**) and after (**B**) embolization as well as 8 wk (**C**) after embolization with coil-gelfoam.

and then became normal in the following 3 d. No obvious changes were found in the volume of the right lobe before and after embolization (Table 1). Contrast-enhanced CT scanning revealed that the left branch of portal vein was recanalized (Figure 2). Ten dogs were killed 8 wk after embolization, no obvious differences were found between the embolized and nonembolized lobes.

Coil-gelfoam group

One dog died of respiratory failure due to narcosis, the rest had successful surgery (91.7%). After embolization, the left branch was completely embolized while the right branch was patent. Compared to pre-embolization situations, the main portal vein and right portal vein were expanded to a certain extent (Figure 3). The portal vein pressure was 96.5±5.1 mm H_2O, 133.9±10.4 mm H_2O before embolization and 97.0±6.3 mm H_2O, 97.6 ±4.7 mm H_2O after embolization. ALT in experimental dogs increased 2 d after embolization ($P<0.01$), and returned to the pre-embolization level in the following 3 d ($P>0.05$). The right lobes of all the survived dogs after embolization were expanded to varying extent. The liver volume was 344.9+38.3, 374.8+43.0 before embolization and 431.5+50.9, 434.0+50.4 after embolization (Table

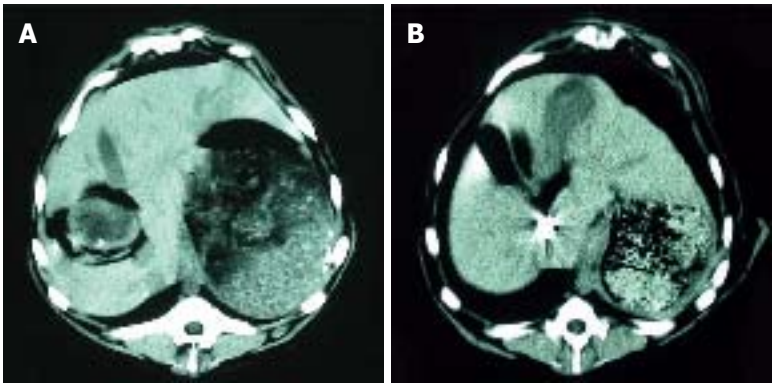

Figure 4 Changes in volume of the right lobe before (**A**) and after (**B**) embolization.

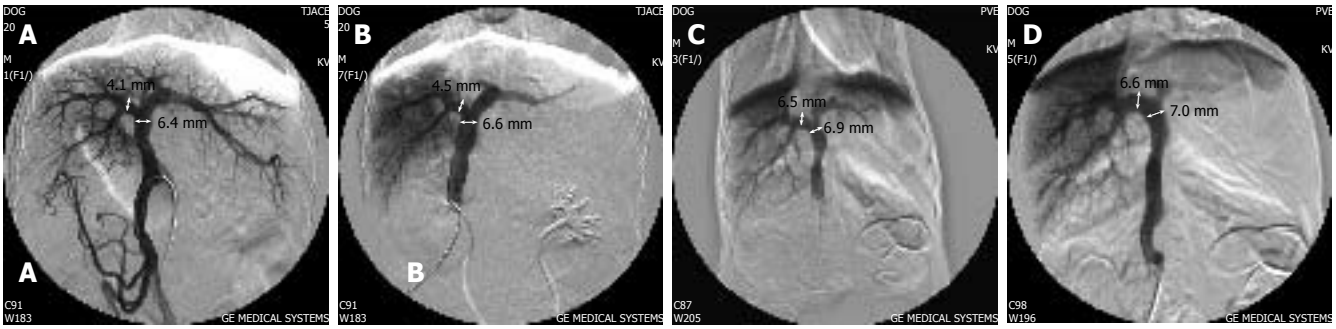

Figure 5 Portal vein angiography before (**A**) and after (**B**) embolization as well as 4 wk (**C**) and 8 wk (**D**) after embolization with absolute ethanol.

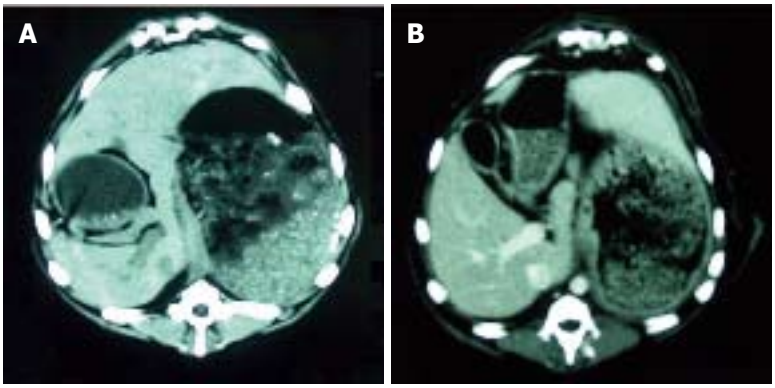

Figure 6 Obvious changes in volume of the right lobe before (**A**) and after (**B**) embolization.

Table 2 Changes of the right lobe volume in coil-gelfoam group (cm^3)

No	Pre-embolization	4W	6W	8W
Dog B1	380	414	474	478
Dog B2	298	325	369	370
Dog B3	376	412	470	472
Dog B4	332	362	417	420
Dog B5	360	392	450	451
Dog B6	294	310	363	365
Dog B7	310	340	379	382
Dog B8	388	426	491	495
Dog B9	300	326	378	385
Dog B10	376	413	473	474
Dog B11	380	403	482	482
Mean±SD	344.9±38.3	374.8±43.0	431.5±50.9	434.0±50.4

Table 3 Changes of the right lobe volume in absolute ethanol group (cm^3)

No.	Pre-embolization	4W	6W	8W
Dog C1	360	392	495	495
Dog C2	272	301	363	363
Dog C3	356	390	492	498
Dog C4	319	350	438	437
Dog C5	329	359	447	449
Dog C6	326	357	430	430
Dog C7	289	293	387	392
Dog C8	328	352	437	439
Dog C9	303	329	412	413
Dog C10	400	409	470	473
Mean±SD	328.2±39.3	353.3±40.3	437.1±45.0	438.9±45.6

2). No further hyperplasia was found (Figure 4). The embolized lobe was dark red with a sharp edge, while the nonembolized lobe was slightly bright red and comparatively plump. Coils were surrounded by fibrin and combined forming the tight emboli. Under microscope, the embolized lobe showed structural changes of hepatic lobules, atrophy and degeneration of liver cells, and congestion of blood vessel, while the nonembolized lobe

revealed cell hypertrophy and obvious hyperplasia.

Absolute ethanol group

One dog died during surgery and 1 dog died in 24 h after surgery due to respiratory failure and acute liver failure caused by the back-flow of absolute ethanol. The rest 10 dogs had successful surgery. The left branch of portal vein was completely embolized, while the right branch was patent. Meanwhile, the main portal vein and right portal vein compared to pre-embolization, were dilated to a certain degree. The left branch of portal vein was occluded without recanalization 8 wk after embolization, while blood flow was seen in the right portal vein and further dilatation was seen in the main portal vein and right portal vein (Figure 5). The portal vein pressure was 95.2±5.2 mm H_2O, 125.2±6.4 mm H_2O before embolization and 96.2± 5.2 mm H_2O, 95.8±6.6 mm H_2O after embolization. ALT was 25.2±1.2, 62.2±3.2, 28.8±1.6, and 26.1±1.3 IU/L after 1, 3, 5, and 7 d of embolization, respectively. ALT values increased by different degrees according to the different injection amount of absolute ethanol, decreased dramatically 3-5 d after embolization and then returned to the pre-embolization level after 1 wk. The volume of right lobe was 328.2±37.1, 353.2±37.9 before embolization and 436.7±42.5, 438.9±45.6 after embolization. No further liver hypertrophy was found (Figure 6). Experimental dogs were killed 8 wk after embolization for pathological observation. The embolized lobe was dark red with a sharp edge, while the nonembolized lobe was slightly bright red and comparatively plump. Under microscope, the embolized lobe showed structural changes of hepatic lobules, liver atrophy, degeneration of liver cells and congestion of fiber tissue, while the nonembolized lobe revealed cell hypertrophy and obvious hyperplasia (Table 3).

DISCUSSION

All our experimental groups with coil-gelfoam and absolute ethanol as embolic agents for left portal vein had right lobe hypertrophy, but the control group and gelfoam group failed to embolize the left portal vein, suggesting that there was no compensatory hypertrophy in the right lobe. The increasing blood flow to the nonembolized portal vein is one of the important factors for liver regeneration. After PVE, dilatation of the nonembolized portal vein branch with obviously increased blood flow could lead to hyperplasia of the nonembolized lobe. These changes were found in our study. Nutritious substances can stimulate regeneration of liver cells. As we know, portal vein blood contains nutritious substances which stimulate regeneration of liver cells. In clinical practice, patients with liver cirrhosis or portal hypertension after shunt operation would suffer from liver atrophy due to decreased blood flow of portal vein. Lindroos *et al*[14] reported that hepatocyte growth factor (HGF) could increase 17 times after two-thirds of rat liver were resected and 13 times after being treated with carbon tetrachloride. Kinoshita *et al*[15] reported that the amount of HGF mRNA in rat liver interstitial cells increased obviously after being treated with carbon tetrachloride, indicating that the increase of HGF can lead to the regeneration of liver cells.

The increase of portal vein pressure and abnormal liver function after PVE, were transient and returned to normal after 4 wk and 1 wk, respectively[16], indicating that transient ischemia of liver portal vein branch has a limited impact on liver function. In our study, five dogs died of accidental extensive embolization of liver portal vein or respiratory failure related to narcosis overdose or absolute ethanol. If PVE is applied in clinical practice, overdose of narcosis can be avoided. The levels of ALT and total bilirubin increased after PVE and returned to normal in 1-2 wk[17,18], demonstrating that the abnormal liver function secondary to PVE is transient and reversible, and does not result in permanent damage to liver function. The portal vein pressure of the three experimental groups was measured at different time points after embolization. We found that the portal vein pressure after embolization was usually higher after embolization, and then returned to normal within 4 wk. Our result is consistent with the study of Baere *et al*[2]. Portal hypertension has not been found in long-term follow-up[19].

All kinds of materials such as absolute ethanol, polydocanol, and gelfoam can be used for portal vein embolization[20-22]. Park *et al*[23] used percutaneous puncture of portal vein to inject Embol into the left branches of pigs' portal vein, and then killed these pigs at different time points to show the safety and effect of Embol. Ko *et al*[24] employed Embol-78 to embolize portal vein of patients with hepatocellular carcinoma or with nonhepatocellular carcinoma to evaluate its effect and safety. Wu *et al*[25] carried out selective embolization experiment of portal vein branches in SD rats with α-cyanoacrylate (DTH) and found compensatory hyperplasia of the nonembolized lobe, suggesting that DTH can effectively induce compensatory hypertrophy of nonembolized lobes. Brown *et al*[26] adopted transcatheter portal vein operation to embolize portal vein branches of liver-cancer patients with polyvinyl alcohol particles (PVA). The above PVA embolization materials have many disadvantages, such as high price, deficient specifications and lack of supply. Gelfoam, coils and absolute ethanol with different characteristics have been used as embolic agents in our clinical practice. Gelfoam can last for a moderate length of time and is safe, non-poisonous and cheap. However, during the process of portal vein embolization, a large amount of gelfoam should be used to completely embolize distal branches of the portal vein, because it easily results in back-flow of gelfoam and accidental embolization of non-target portal vein. We also found that recanalization occurred in gelfoam-embolic portal vein, which failed to effectively induce compensatory hypertrophy of the nonembolized lobe. Therefore, it is not suitable and safe to use gelfoam as an embolic agent for PVE. On the other hand, after being inserted into the target blood vessel, coils lead to mechanical embolization and neointimal hyperplasia, forming permanent embolization. However, coils cannot embolize small blood vessels and easily result in collateral vessels, limiting its use as a PVE embolic agent for inducing hypertrophy of liver. Therefore, combination of gelfoam and coils is a kind of safe and effective mode to achieve complete embolization and to avoid displacement and accidental embolization. In addition, absolute ethanol

can instantly produce permanent embolization in target blood vessels without collateral vessel formation after embolization, thus preventing recanalization of blood vessels and achieving better embolized results. The above results show that absolute ethanol is better than coil-gelfoam for PVE. However, when absolute ethanol is used as an embolic agent, back-flow easily occurs. Once back-flow occurs, it results in serious consequences. It is safe and effective to settle back-flow of absolute ethanol using a balloon catheter[27,28]. In absolute ethanol group, two dogs died of injection of large absolute ethanol doses, while the rest of the dogs which received an injection of small absolute ethanol doses survived. A larger amount of absolute ethanol can achieve good embolic effect, but death rate of experimental animals increases. A smaller amount of absolute ethanol cannot achieve satisfactory results. Thus, further research is needed to determine the safe amount of absolute ethanol injected. Absolute ethanol is a peripheral embolic agent. Compared to coil-gelfoam, it can reach distal branches of portal vein. After embolization, collateral vessels are difficult to form. At the same time, absolute ethanol exerts stronger influence on embolized portal vein branches and even smaller distal branches. Absolute ethanol causes certain damage to neointimal cells inside small branches of hepatic arteries and hepatic cells. The event enhances embolic effect, prevents recanalization of blood vessel, thus stimulating and inducing compensatory hypertrophy of nonembolized lobes more significantly.

In conclusion, gelfoam alone cannot effectively induce compensatory hypertrophy. Coil-gelfoam and absolute ethanol can be used as selective embolic agents to induce compensatory hypertrophy of liver, but absolute ethanol is better than coil-gelfoam for inducing compensatory hypertrophy. However, the safety of absolute ethanol is inferior to that of coil-gelfoam.

REFERENCES

1 **Hemming AW**, Reed AI, Howard RJ, Fujita S, Hochwald SN, Caridi JG, Hawkins IF, Vauthey JN. Preoperative portal vein embolization for extended hepatectomy. *Ann Surg* 2003; **237**: 686-91; discussion 691-3

2 **de Baere T**, Roche A, Vavasseur D, Therasse E, Indushekar S, Elias D, Bognel C. Portal vein embolization: utility for inducing left hepatic lobe hypertrophy before surgery. *Radiology* 1993; **188**: 73-77

3 **Kawasaki S**, Imamura H, Kobayashi A, Noike T, Miwa S, Miyagawa S. Results of surgical resection for patients with hilar bile duct cancer: application of extended hepatectomy after biliary drainage and hemihepatic portal vein embolization. *Ann Surg* 2003; **238**: 84-92

4 **Belghiti J**. Arguments for a selective approach of preoperative portal vein embolization before major hepatic resection. *J Hepatobiliary Pancreat Surg* 2004; **11**: 21-24

5 **Liu H**, Fu Y. Portal vein embolization before major hepatectomy. *World J Gastroenterol* 2005; **11**: 2051-2054

6 **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

7 **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

8 **Madoff DC**, Hicks ME, Abdalla EK, Morris JS, Vauthey JN. Portal vein embolization with polyvinyl alcohol particles and coils in preparation for major liver resection for hepatobiliary malignancy: safety and effectiveness--study in 26 patients. *Radiology* 2003; **227**: 251-260

9 **Di Stefano DR**, de Baere T, Denys A, Hakime A, Gorin G, Gillet M, Saric J, Trillaud H, Petit P, Bartoli JM, Elias D, Delpero JR. Preoperative percutaneous portal vein embolization: evaluation of adverse events in 188 patients. *Radiology* 2005; **234**: 625-630

10 **Elias D**, Debaere T, Roche A, Bonvallot S, Lasser P. Preoperative selective portal vein embolizations are an effective means of extending the indications of major hepatectomy in the normal and injured liver. *Hepatogastroenterology* 1998; **45**: 170-177

11 **Tominaga M**, Ku Y, Iwasaki T, Fukumoto T, Muramatsu S, Kusunoki N, Suzuki Y, Fujino Y, Kuroda Y. Effect of portal vein embolization on function of the nonembolized lobes of the liver: Evaluation by first-pass hepatic lidocaine extraction in dogs. *Surgery* 2002; **132**: 424-430

12 **Duncan JR**, Hicks ME, Cai SR, Brunt EM, Ponder KP. Embolization of portal vein branches induces hepatocyte replication in swine: a potential step in hepatic gene therapy. *Radiology* 1999; **210**: 467-477

13 **Kaido T**, Yoshikawa A, Seto S, Yamaoka S, Sato M, Ishii T, Imamura M. Portal branch ligation with a continuous hepatocyte growth factor supply makes extensive hepatectomy possible in cirrhotic rats. *Hepatology* 1998; **28**: 756-760

14 **Lindroos PM**, Zarnegar R, Michalopoulos GK. Hepatocyte growth factor (hepatopoietin A) rapidly increases in plasma before DNA synthesis and liver regeneration stimulated by partial hepatectomy and carbon tetrachloride administration. *Hepatology* 1991; **13**: 743-750

15 **Kinoshita T**, Tashiro K, Nakamura T. Marked increase of HGF mRNA in non-parenchymal liver cells of rats treated with hepatotoxins. *Biochem Biophys Res Commun* 1989; **165**: 1229-1234

16 **Tanaka H**, Kinoshita H, Hirohashi K, Kubo S, Lee KC. Increased safety by two-stage hepatectomy with preoperative portal vein embolization in rats. *J Surg Res* 1994; **57**: 687-692

17 **Lee KC**, Kinoshita H, Hirohashi K, Kubo S, Iwasa R. Extension of surgical indications for hepatocellular carcinoma by portal vein embolization. *World J Surg* 1993; **17**: 109-115

18 **Zhan SH**, Han Hongjie, Wang Guoliang, Kan Zx. Experimental study of interventional embolization of right portal vein branch on rats. *Jieru Fangshexue Zazhi* 2001; **10**: 167-170

19 **Burgener FA**, Gutierrez OH, Logsdon GA. Angiographic, hemodynamic, and histologic evaluation of portal hypertension and periportal fibrosis induced in the dog by intraportal polyvinyl alcohol injections. *Radiology* 1982; **143**: 379-385

20 **Ogasawara K**, Uchino J, Une Y, Fujioka Y. Selective portal vein embolization with absolute ethanol induces hepatic hypertrophy and makes more extensive hepatectomy possible. *Hepatology* 1996; **23**: 338-345

21 **Yamakado K**, Takeda K, Nishide Y, Jin J, Matsumura K, Nakatsuka A, Hirano T, Kato N, Nakagawa T. Portal vein embolization with steel coils and absolute ethanol: a comparative experimental study with canine liver. *Hepatology* 1995; **22**: 1812-1818

22 **Kaneko T**, Nakao A, Takagi H. Experimental studies of new embolizing material for portal vein embolization. *Hepatogastroenterology* 2000; **47**: 790-794

23 **Park S**, Yoon HK, Lee N, Huh SJ, Kang GH, Lee I, Sung KB, Song HY. Portal vein embolization with use of a new liquid embolic material: an experimental study. *J Vasc Interv Radiol* 1999; **10**: 339-345

24 **Ko GY**, Sung KB, Yoon HK, Kim JH, Weon YC, Song HY. Preoperative portal vein embolization with a new liquid embolic agent. *Radiology* 2003; **227**: 407-413

25 **Wu XF**, Huan J, Ling ZY, Ji Y. Experimental study of selectivity embolization of portal vein on rats. *Zhongguo Weixunhuan* 2000; **4**: 218-220

26 **Brown KT**, Brody LA, Decorato DR, Getrajdman GI. Portal vein embolization with use of polyvinyl alcohol particles. *J Vasc Interv Radiol* 2001; **12**: 882-886

27 **Satake M**, Tateishi U, Kobayashi T, Murata S, Kumazaki T. Percutaneous transhepatic portal vein embolization: effectiveness of absolute ethanol infusion with balloon catheter in a pig model. *Acta Radiol* 2005; **46**: 344-352
28 **Kim MJ**, Choo SW, Do YS, Park KB, Han YH, Choo IW, Cho JM, Cho JW, Kim SJ, Sohn TS. Use of double-occlusion balloon catheter: preoperative portal vein embolization for induction of future remnant liver hypertrophy. *Cardiovasc Intervent Radiol* ; **27**: 16-20
29 **Lu MD**, Liang LJ, Huang JF, Ye WJ, Yang QS, Peng BG, Xie XY. Portal vein embolization with ethanol injection via a fine needle in dogs. *Surg Today* 1995; **25**: 416-420

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BASIC RESEARCH

Analysis of p53 and vascular endothelial growth factor expression in human gallbladder carcinoma for the determination of tumor vascularity

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Abstract

AIM: To examine the expression of p53 and vascular endothelial growth factor (VEGF) as well as microvessel count (MVC) and to investigate the role of VEGF as an angiogenic marker and the possible role of p53 in the regulation of angiogenesis in human gallbladder carcinoma.

METHODS: Surgically resected specimens of 49 gallbladder carcinomas were studied by immunohistochemical staining for p53 protein, VEGF, and factor VIII-related antigen. VEGF expression and mutant p53 expression were then correlated with Nevin stage, differentiation grade, MVC, and lymph node metastasis.

RESULTS: Positive p53 protein and VEGF expressions were found in 61.2% and 63.3% of tumors, respectively. p53 and VEGF staining status was identical in 55.1% of tumors. The Nevin staging of p53- or VEGF-positive tumors was significantly later than that of negative tumors. The MVC in p53- or VEGF-positive tumors was significantly higher than that in negative tumors, and MVC in both p53- and VEGF-negative tumors was significantly lower than that in the other subgroups.

CONCLUSION: Our findings suggest that p53-VEGF pathway can regulate tumor angiogenesis in human gallbladder carcinoma. Combined analysis of p53 and VEGF expression might be useful for predicting the tumor vascularity of gallbladder cancer.

Key words: Gallbladder neoplasms; Protein p53; Neovascularization; Pathology

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INTRODUCTION

Angiogenesis refers to the formation of new blood vessels from a pre-existing vascular network. Adequate vascularization is critically required for the growth and metastasis of human solid tumors[1-5]. Angiogenesis is influenced by multiple factors such as growth factors, extracellular matrix proteins, and cell adhesion molecules, as well as by the imbalance between angiogenesis-stimulating factors and inhibiting factors[6-10]. One of the most important angiogenesis-stimulating factors is VEGF, a diffusible endothelial cell-specific mitogen that induces endothelial-cell proliferation and increases vascular permeability. There is evidence that it may promote a degradative environment that facilitates migration of endothelial cells by the conduction of plasminogen activators and collagenase[11]. VEGF plays a major role in regulating angiogenesis. Most tumors produce high levels of VEGF. Neutralization of VEGF leads to a marked inhibition of angiogenesis and tumor growth[12-15].

However, there is little information about the genetic changes associated with angiogenesis. So far, most studies have stressed the role of oncogene activation and tumor suppressor gene (TSG) inactivation in promoting aberrant tumor-cell proliferation. Although it remains unclear whether any of these genetic alterations can trigger the disruption of control of angiogenesis, some *in vitro* studies have demonstrated the important role played by the p53 tumor suppressor gene in controlling tumor angiogenesis[16,17]. In the present study, we have examined p53 and VEGF expressions as well as microvessel count (MVC) in human gallbladder carcinoma tissues to investigate the involvement of the p53 gene in regulation of tumor angiogenesis and its clinical significance.

Table 1 Nevin staging system for gallbladder cancer[18]

Stage	Definitions
1	Tumor invades mucosa only
2	Tumor invades muscularis and mucosa
3	Tumor invades subserosa, muscularis, and mucosa
4	Tumor invades all layers of gallbladder wall plus cystic lymph node
5	Tumor extension into liver bed or distant spread

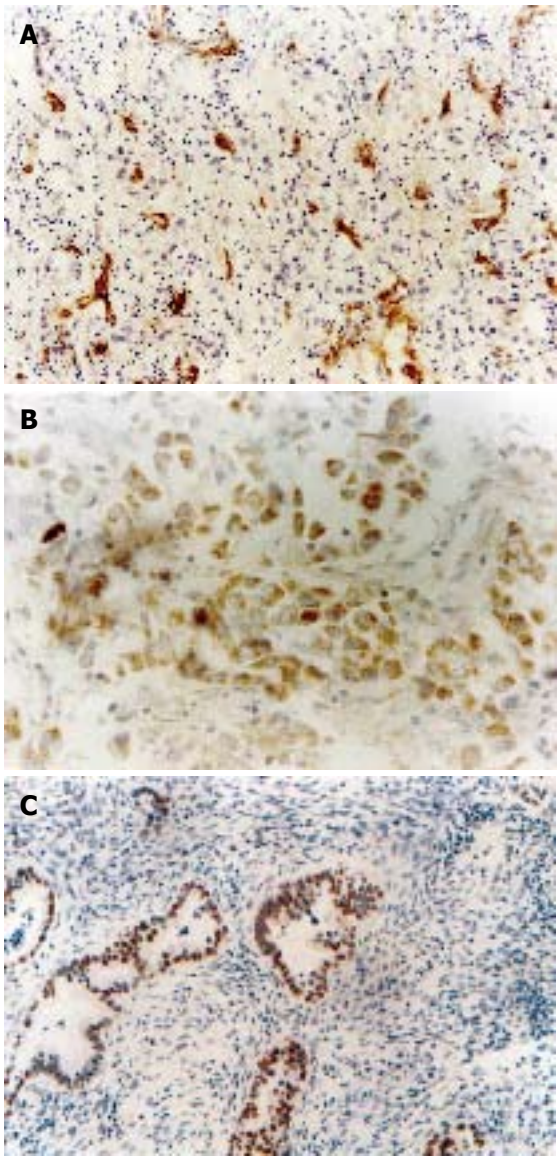

Figure 1 Microvessel distribution (**A**) and expression of VEGF (**B**) and p53 (**C**) in gallbladder carcinoma tissue.

MATERIALS AND METHODS

Clinical materials

Forty-nine histologically proven gallbladder carcinomas were selected. All patients were surgically treated at the Department of General Surgery of the First and Second Hospitals affiliated to China Medical University, Shenyang, China, but did not receive chemotherapy or anti-angiogenesis therapy before surgery. The cases included 24 males and 25 females. The average age of the males and females was 62 years and 55 years, respectively. Six cases had papillary adenocarcinoma (12.2%), 43 cases had tubular adenocarcinoma (87.8%), 22 cases had well-differentiated tumor (44.9%), 17 cases had moderately differentiated tumor (34.7%), 10 cases had poorly differentiated tumor (20.4%). Nevin stage (Table 1) was determined based on clinical materials: 19 cases of S1, S2, and S3, and 30 cases of S4 and S5. Twenty-seven cases (55.1%) had lymph node metastasis (+), 22 cases (44.9%) had no lymph node metastasis (-). In each case, all available sections stained with hematoxylin and eosin were reviewed.

Immunohistochemical study of p53 and VEGF

Four micrometer thick sections from the formalin-fixed and paraffin-embedded tissues were placed on the poly-L-lysine-coated slides for immunohistochemistry.

Immunohistochemical staining was performed by the streptoavidin-biotin method. In brief, sections were de-paraffinized and incubated with 3% hydrogen peroxide for 20 min to block endogenous peroxidase activity. The sections were treated twice with microwave at 500 W for 5 min each time in 10 mmol/L sodium citrate (pH 6.0). After washing with PBS, the sections were incubated in 10% normal rabbit or goat serum for 20 min to reduce non-specific antibody binding. The antibodies used were mouse monoclonal antibody (MAb) against human p53 protein (Maxin-Bio Co., Fuzhou, China) in 1:100 dilution at 4 ℃ overnight, and a rabbit polyclonal antibody against human VEGF (A-20; Santa Cruz Biotechnology, Santa Cruz, CA, USA) in 1:50 dilution at 4 ℃ overnight. After washing thrice with PBS, the sections were incubated with biotinylated rabbit anti-mouse or goat anti-rabbit immunoglobulin G (Maxin-Bio Co., Fuzhou, China) for 30 min, washed thrice again with PBS, treated with streptavidin-peroxidase reagent for 30 min and then washed thrice with PBS again. Finally, the specimens were incubated in PBS containing diaminobenzidine and 1% hydrogen peroxide for 5 min and counterstained with hematoxylin. PBS was substituted for each primary antibody as negative control. Slides were examined by two investigators without the knowledge of the corresponding clinicopathologic data. p53 immunoreactivity was assessed as being positive only when tumors exhibited intense nuclear staining, and reactivity was categorized into negative expression (less than 10% positive tumor cells) and positive expression (at least 10% positive tumor cells). Immunostaining for VEGF was considered positive when unequivocal staining of cell membrane or cytoplasm was observed in more than 10% of tumor cells.

Microvessel staining and counting

Microvessel staining and counting were performed as described previously[19]. Briefly, intratumoral microvessels were highlighted by immunostaining with a mouse MAb against factor VIII-related antigen (F-VIII RAg) (Maxin-Bio Co., Fuzhou, China) in 1:100 dilution and incubated at 4 ℃ overnight, after pre-digestion with 0.1% (v/v) trypsin at 37 ℃ for 20 min. Any single brown-stained cell or cluster of endothelial cells that was clearly separated from adjacent vessels, tumor cells and other connective tissues was considered as a microvessel. The stained sections were screened at ×40 fields to identify the regions of the high-

Table 2 Clinicopathologic characteristics of MVC in gallbladder carcinoma (mean ± SD)

Characteristics	*n*	MVC
Tumor differentiation		
Good	22	30±11
Moderate-poor	27	38±11[a]
Nevin staging		
S1, S2, S3	19	26±11
S4, S5	30	40±8[b]
Lymph node metastasis		
Yes	27	40±10
No	22	28±11[d]

[a]*P*<0.05 *vs* good group; [b]*P*<0.01 *vs* "S1, S2, S3" group; [d]*P*<0.01 *vs* yes group.

Table 3 Clinicopathologic characteristics of VEGF expression in gallbladder carcinoma

Characteristics	*n*	+	%
Tumor differentiation			
Good	22	15	68.2
Moderate-poor	27	16	59.3
Nevin staging			
S1, S2, S3	19	8	42.1
S4, S5	30	23	76.7[a]
Lymph node metastasis			
Yes	27	21	77.8
No	22	12	54.5[c]

[a]*P*<0.05 *vs* "S1, S2, S3" group; [c]*P*<0.05 *vs* yes group.

Table 4 Clinicopathologic characteristics of mutant p53 expression in gallbladder carcinoma

Characteristics	*n*	+	%
Tumor differentiation			
Good	22	13	59.1
Moderate-poor	27	17	63.0
Nevin staging			
S1, S2, S3	19	9	47.4
S4, S5	30	21	70.0[a]
Lymph node metastasis			
Yes	27	20	74.1
No	22	10	45.5[c]

[a]*P*<0.05 *vs* "S1, S2, S3" group; [c]*P*<0.05 *vs* yes group.

Table 5 Relationship between MVC and presence of p53 and VEGF (mean ± SD, *n* (%))

	Patients,	MVC	
	VEGF(+)	VEGF(-)	Total
p53(+)	27 (55.1%)	3 (6.1%)	30 (61.2%)
	41±9	35±10	36±10
p53(-)	4 (8.2%)	15 (30.6%)	19 (38.8%)
	36±12	28±13[a]	30±12[e]
Total	31 (63.3%)	18 (36.7%)	49 (100%)
	37±11	30±12[c]	

[a]*P*<0.05 *vs* others, [c]*P*<0.05 *vs* "VEGF(+)" group, [e]*P*<0.05 *vs* "p53(+)" group.

est vascular density within the tumor. Vessels were counted in the five regions of the highest vascular density at ×200 fields (Olympus BH-2 microscope, 0.74 mm^2 per field). MVC was the mean number of vessels in these areas.

Statistical analysis

The relationship between p53 or VEGF expression and MVC was evaluated by *t*-test, and the relationship between p53 and VEGF expression and various clinicopathologic factors was examined by the χ^2 test. $P<0.05$ was considered statistically significant.

RESULTS

Expression of VEGF, p53, and MVC

The microvessels in malignant tissues were heterogeneously distributed. These neovascular areas occurred anywhere within the tumor but most frequently at the tumor margins (Figure 1A). The immunoreactive regions of VEGF were located in cytoplasms or membranes of the gallbladder carcinoma cells (Figure 1B). The immunoreactive regions of mutant p53 were located in the nuclei of the gallbladder carcinoma cells (Figure 1C).

Clinicopathologic characteristics of MVC, VEGF, and p53 expressions

The average MVC in 49 cases of gallbladder carcinoma was (35±12)/HP. MVC was markedly higher in cases of Nevin stage S4-S5 than in those of S1-S3 ($P<0.01$). MVC in moderate-poor differentiation group was higher than that in good differentiation group ($P<0.05$). MVC in patients with lymph node metastasis was significantly higher than that in those without lymph node metastasis ($P<0.01$, Table 2).

The positive rate of VEGF expression was 63.3% in these 49 cases and was higher in cases of Nevin stage S4-S5 (76.7%) than in those of S1-S2 (42.1%) ($P<0.05$). The positive rate of VEGF expression was not correlated with tumor differentiation ($P>0.05$). With regard to the association with lymph node metastases, the positive rate of VEGF expression was significantly higher in patients with metastasis than in those without metastasis ($P<0.05$, Table 3).

The positive rate of mutant p53 expression was 61.2% in these 49 cases and was lower in cases of Nevin stage S1-S3 (47.4%) than in those of S4-S5 (70.0%), the difference was statistically significant ($P<0.05$). The positive rate of mutant p53 expression was not correlated with tumor differentiation ($P>0.05$). With regard to the association with lymph node metastases, the positive rate of p53 expression was significantly higher in patients with metastasis than in those without metastasis ($P<0.05$, Table 4).

Correlation between p53, VEGF, and MVC

Table 5 shows the relationships between MVC and the presence of p53 and VEGF. Staining status was identical in 27 of 49 tumors (55.1%), and a significant ($P<0.05$) association between p53 and VEGF expression was demonstrated. The MVC in tumors that were p53 or VEGF positive was significantly ($P<0.05$) higher than that in p53- or VEGF-negative tumors. Moreover, the

mean MVC in tumors of all the subgroups that were both p53 and VEGF positive was the highest, while the mean MVC was significantly lower in patients with tumors that were both p53 and VEGF negative than in all the other subgroups.

DISCUSSION

In 1971, Folkman proposed that tumor growth depends on angiogenesis. Now there is considerable indirect and direct evidence that tumor growth is dependent on angiogenesis. Numerous studies showed that neovascularization is closely associated with growth, invasion, metastasis, staging and prognosis of tumors[7,20-23]. Our study also indicated that angiogenesis was correlated with the Nevin staging and tumor differentiation. The higher the staging is, the poorer the differentiation and the higher the level of MVC are in gallbladder carcinoma. Since MVC is of prognostic value, we investigated whether this holds true for VEGF expression as well. Our findings demonstrate that both VEGF expression and MVC are significantly elevated in patients with disease progression and depend on each other. Tumors with established poor outcome (staging, MVC) are likely to produce higher levels of VEGF, suggesting that VEGF significantly contributes to the poor outcome in these patients.

The most common genetic alteration in various cancers is loss of the p53 tumor suppressor gene function. P53 protein has various important functions in cellular integration, including cell growth control, response to DNA damage, checkpoint mechanisms during the cell cycle, regulation of transcription and control of genomic stability. It is suggested that p53 protein may play a role in suppressing angiogenesis[24-27]. In addition, it was reported that a pathway from p53 regulating VEGF induces angiogenesis in cellular transfection models[16]. In the present study, we explored the possibility of a relationship between aberrant p53 and VEGF expression and tumor angiogenesis in gallbladder carcinoma. We observed that VEGF-positive tumors had significantly more microvessels than VEGF-negative tumors. Our findings agree with other studies[13,28,29], suggesting that VEGF-induced tumor angiogenesis plays an important role not only in tumor progression but also in metastasis. Although little is known concerning the VEGF regulatory pathways, Kieser *et al*.[16] reported that mutant-type p53 might stimulate VEGF expression. In the present study, p53 and VEGF expression status coincided in 27 of 49 (55.1%) tumors, and a significant association was found between the two factors. Moreover, MVC was the highest in p53 and VEGF positive tumors and the lowest in p53 and VEGF negative tumors. These results suggest not only the existence of a p53-VEGF regulatory pathway in gallbladder carcinomas, but also a possible role of such a pathway in regulating tumor angiogenesis in this type of cancer. These further suggest that tumors that have lost p53 suppressor function not only permit uncontrolled cell growth, but also possibly produce a suitable environment for hematogenous metastasis by stimulating VEGF-induced angiogenesis in the primary site of carcinoma. In contrast, tumors with preserved p53 function and angiogenic inhibitory pathway are less possible to progress, and the p53 gene may therefore contribute to better prognosis.

In conclusion, our results demonstrate that abnormal p53 accumulation is closely associated with VEGF expression and tumor vascularity in human gallbladder carcinoma. P53 plays a critical role in suppressing tumor growth by regulating tumor angiogenesis. Clinical application of combined analysis of p53 and VEGF expression may be useful for predicting the clinical staging, occurrence of metastasis in patients with this disease. More prospective studies should be made to prove our findings.

REFERENCES

1 **Weidner N**, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis--correlation in invasive breast carcinoma. *N Engl J Med* 1991; **324**: 1-8

2 **Macchiarini P**, Fontanini G, Hardin MJ, Squartini F, Angeletti CA. Relation of neovascularisation to metastasis of non-small-cell lung cancer. *Lancet* 1992; **340**: 145-146

3 **Liu XP**, Song SB, Li G, Wang DJ, Zhao HL, Wei LX. Correlations of microvessel quantitation in colorectal tumors and clinicopathology. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 37-39

4 **Gao GL**, Yang Y, Yang SF, Ren CW. Relationship between proliferation of vascular endothelial cells and gastric cancer. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 282-284

5 **Jia L**, Chen TX, Sun JW, Na ZM, Zhang HH. Relationship between microvessel density and proliferating cell nuclear antigen and prognosis in colorectal cancer. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 74-76

6 **Liotta LA**, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell* 1991; **64**: 327-336

7 **Song ZJ**, Gong P, Wu YE. Relationship between the expression of iNOS,VEGF,tumor angiogenesis and gastric cancer. *World J Gastroenterol* 2002; **8**: 591-595

8 **Liu H**, Wu JS, Li LH, Yao X. The expression of platelet-derived growth factor and angiogenesis in human coloreactal carcinoma. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 661-664

9 **Ng IO**, Poon RT, Lee JM, Fan ST, Ng M, Tso WK. Microvessel density, vascular endothelial growth factor and its receptors Flt-1 and Flk-1/KDR in hepatocellular carcinoma. *Am J Clin Pathol* 2001; **116**: 838-845

10 **Joo YE**, Sohn YH, Joo SY, Lee WS, Min SW, Park CH, Rew JS, Choi SK, Park CS, Kim YJ, Kim SJ. The role of vascular endothelial growth factor (VEGF) and p53 status for angiogenesis in gastric cancer. *Korean J Intern Med* 2002; **17**: 211-219

11 **Ferrara N**, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem Biophys Res Commun* 1989; **161**: 851-858

12 **Fidler IJ**, Ellis LM. The implications of angiogenesis for the biology and therapy of cancer metastasis. *Cell* 1994; **79**: 185-188

13 **Kondo Y**, Arii S, Furutani M, Isigami S, Mori A, Onodera H, Chiba T, Imamura M. Implication of vascular endothelial growth factor and p53 status for angiogenesis in noninvasive colorectal carcinoma. *Cancer* 2000; **88**: 1820-1827

14 **Wizigmann-Voos S**, Plate KH. Pathology, genetics and cell biology of hemangioblastomas. *Histol Histopathol* 1996; **11**: 1049-1061

15 **Arii S**, Mori A, Uchida S, Fujimoto K, Shimada Y, Imamura M. Implication of vascular endothelial growth factor in the development and metastasis of human cancers. *Hum Cell* 1999; **12**: 25-30

16 **Kieser A**, Weich HA, Brandner G, Marmé D, Kolch W. Mutant p53 potentiates protein kinase C induction of vascular endothelial growth factor expression. *Oncogene* 1994; **9**: 963-969

17 **Volpert OV**, Dameron KM, Bouck N. Sequential development of an angiogenic phenotype by human fibroblasts progressing

to tumorigenicity. *Oncogene* 1997; **14**: 1495-1502
18 **Nevin JE**, Moran TJ, Kay S, King R. Carcinoma of the gallbladder: staging, treatment, and prognosis. *Cancer* 1976; **37**: 141-148
19 **Tian Y**, Zhu LL, Guo RX, Fan CF. Correlation of P-glycoprotein expression with poor vascularization in human gallbladder carcinomas. *World J Gastroenterol* 2003; **9**: 2817-2820
20 **Niedergethmann M**, Hildenbrand R, Wostbrock B, Hartel M, Sturm JW, Richter A, Post S. High expression of vascular endothelial growth factor predicts early recurrence and poor prognosis after curative resection for ductal adenocarcinoma of the pancreas. *Pancreas* 2002; **25**: 122-129
21 **Niemöller K**, Jakob C, Heider U, Zavrski I, Eucker J, Kaufmann O, Possinger K, Sezer O. Bone marrow angiogenesis and its correlation with other disease characteristics in multiple myeloma in stage I versus stage II-III. *J Cancer Res Clin Oncol* 2003; **129**: 234-238
22 **Takahashi R**, Tanaka S, Kitadai Y, Sumii M, Yoshihara M, Haruma K, Chayama K. Expression of vascular endothelial growth factor and angiogenesis in gastrointestinal stromal tumor of the stomach. *Oncology* 2003; **64**: 266-274
23 **Onogawa S**, Tanaka S, Oka S, Morihara M, Kitadai Y, Sumii M, Yoshihara M, Shimamoto F, Haruma K, Chayama K. Clinical significance of angiogenesis in rectal carcinoid tumors. *Oncol Rep* 2002; **9**: 489-494
24 **Mazurek A**, Pierzyński P, Kuć P, Kopinski P, Terlikowski S, Niklińska W, Trojan J, Laudański T. Evaluation of angiogenesis, p-53 tissue protein expression and serum VEGF in patients with endometrial cancer. *Neoplasma* 2004; **51**: 193-197
25 **Nayak A**, Ralte AM, Sharma MC, Singh VP, Mahapatra AK, Mehta VS, Sarkar C. p53 protein alterations in adult astrocytic tumors and oligodendrogliomas. *Neurol India* 2004; **52**: 228-232
26 **Liu J**, Shibata T, Qu R, Ogura M, Hiraoka M. Influences of the p53 status on hypoxia-induced gene expression. *J Radiat Res* 2004; **45**: 333-339
27 **Cao W**, Chen X, Dai H, Wang H, Shen B, Chu D, McAfee T, Zhang ZF. Mutational spectra of p53 in geographically localized esophageal squamous cell carcinoma groups in China. *Cancer* 2004; **101**: 834-844
28 **Takahashi Y**, Bucana CD, Cleary KR, Ellis LM. p53, vessel count, and vascular endothelial growth factor expression in human colon cancer. *Int J Cancer* 1998; **79**: 34-38
29 **Kang SM**, Maeda K, Onoda N, Chung YS, Nakata B, Nishiguchi Y, Sowa M. Combined analysis of p53 and vascular endothelial growth factor expression in colorectal carcinoma for determination of tumor vascularity and liver metastasis. *Int J Cancer* 1997; **74**: 502-507

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BASIC RESEARCH

Effect of genistein on voltage-gated potassium channels in guinea pig proximal colon smooth muscle cells

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Abstract

AIM: To investigate the action of genistein (GST), a broad spectrum tyrosine kinase inhibitor, on voltage-gated potassium channels in guinea pig proximal colon smooth muscle cells.

METHODS: Smooth muscle cells in guinea pig proximal colon were enzymatically isolated. Nystatin-perforated whole cell patch clamp technique was used to record potassium currents including fast transient outward current (I_{Kto}) and delayed rectifier current (I_{Kdr}), two of which were isolated pharmacologically with 10 mmol/L tetraethylammonium or 5 mmol/L 4-aminopyridine. Contamination of calcium-dependent potassium currents was minimized with no calcium and 0.2 mmol/L $CdCl_2$ in an external solution.

RESULTS: GST (10-100 μmol/L) reversibly and dose-dependently reduced the peak amplitude of I_{Kto} with an IC_{50} value of 22.0±6.9 μmol/L. To a lesser extent, I_{Kdr} was also inhibited in both peak current and sustained current. GST could not totally block the outward potassium current as a fraction of the outward potassium current, which was insensitive to GST. GST had no effect on the steady-state activation ($n = 6$) and inactivation kinetics ($n = 6$) of I_{Kto}. Sodium orthovanadate (1 mmol/L), a potent inhibitor of tyrosine phosphatase, significantly inhibited GST-induced inhibition ($P < 0.05$).

CONCLUSION: GST can dose-dependently and reversibly block voltage-gated potassium channels in guinea pig proximal colon smooth muscle cells.

Key words: Protein tyrosine kinase; Patch-clamp technique; Genistein; Voltage-gated potassium channel; Colon; Smooth muscle cells

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INTRODUCTION

Ion channels are targets of many intracellular signaling pathways, including protein phosphorylation and dephosphorylation. These processes can modify channel activity and dramatically alter the electrophysiological properties of both excitable and nonexcitable cells[1]. In addition to the extensive information available about the regulation of ion channels by serine-threonine kinases, an emerging body of evidence suggests that channels are also regulated by phosphorylation on tyrosine residues, such as ligand-gated channels AChR[2], NMDA receptor[3], potassium channels[4,5] as well as calcium channels[6,7]. GST, a specific inhibitor of tyrosine kinases, inhibits visceral smooth muscle contraction induced by tyrosine phosphatase inhibitor vanadate[8], angiotensin II[9] and carbachol[10] via tyrosine kinase-mediated process. Potassium channels, which are substrates for protein phosphorylation and dephosphorylation, control the contraction of gastrointestinal smooth muscles by setting resting potential and influencing slow waves and action potential configuration[11]. However, little is known of the modulation of potassium channels in gastrointestinal smooth muscle cells via tyrosine kinase pathway. This study was to investigate the effects and mechanisms of GST on voltage-gated potassium channels (Kv) in smooth muscle cells of guinea pig proximal colon.

MATERIALS AND METHODS

Cell dissociation

Smooth muscle cells were enzymatically isolated using modified procedures as previously described[12]. Briefly, male guinea pigs (200-350 g) were killed by cervical dislocation and proximal colon about 2 cm aboral to cecum was rapidly excised. Under anatomical microscope, smooth muscle strips were dissected out, cut into small pieces and incubated for 30 min in low calcium solution containing 10 mmol/L HEPES, 135 mmol/L NaCl, 6 mmol/L KCl, 0.05 mmol/L $CaCl_2$, 1.2 mmol/L $MgCl_2$, 10 mmol/L Glucose, pH 7.4. The pieces of muscles were then transferred to low calcium solution containing

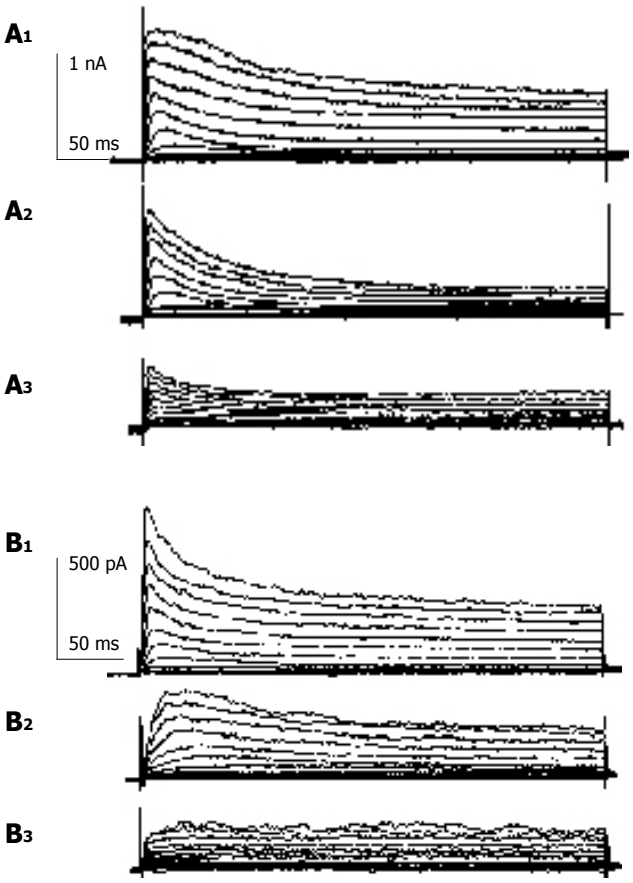

Figure 1 Two components of voltage-gated potassium currents in guinea pig colon smooth muscle cells and effect of GST. **A1**-**A3**: Currents recorded every 10 s between -80 and +50 mV then back to -40 mV with holding potential -80 mV in the control (**A1**), in the presence of 10 mmol/L TEA (**A2**) and TEA and 30 μmol/ GST (**A3**). **B1**: Currents recorded every 10 s between -80 and +50 mV with holding potential -80 mV in the control; **B2**: current in the presence of 5 mmol/L 4-AP developed slowly and showed slower inactivation over 400 ms; **B3**: effect of 50 μmol/L GST in the presence of 5 mmol/L 4-AP.

3 g/L papain, 2 g/L DTT, 2 g/L bovine serum albumin. Tissues were incubated at 36 ℃ in enzyme solution for 15 min and then suspended in enzyme-free low calcium solution. Tissue pieces were gently agitated to create a cell suspension. Dispersed cells were stored at 4 ℃ for later use. Experiments were performed at 20-22 ℃ within 10 h.

Perforated whole cell voltage clamp recording

Relaxed single colon smooth muscle cells with smooth appearance and spindle shape observed under an inverted microscope (IX70, Olympus) were used. Myocytes were perfused with Ca^{2+}-free cell bath solution in a self-made small volume chamber in which solution could be exchanged in 30 s. The composition of bath solution was the same as that of low calcium solution except for exclusion of calcium and inclusion of 0.2 mmol/L $CdCl_2$ to minimize the contamination of calcium-dependent potassium currents.

Patch clamp micropipettes were pulled with a programmable puller (P-97, Sutter Instruments) and their tips were fire polished (CPM-2, ALA Co.). The pipette resistance was 3-5 MΩ. Currents were amplified with Axopatch 200B (Axon Instruments). Data were filtered at 1 kHz and analyzed with pClamp software (version 8.2). Nystatin-perforated whole cell patch clamp technique was used to record voltage-gated potassium currents. Nystatin was dissolved in DMSO at a concentration of 25 g/L, and then added to the internal pipette solution to yield a final nystatin concentration of 100 mg/L. The internal solution contained 10 mmol/L HEPES, 110 mmol/L glucoante (potassium salt), 30 mmol/L KCl, 10 mmol/L NaCl, 2 mmol/L $MgCl_2$, pH 7.2. After giga seals were obtained, access resistance was monitored for 10 min to allow the drop of access resistance (average 17.7 ± 5.7 MΩ, n = 28) and then compensated at 70%. Macroscopic current values were normalized for cell capacitance as whole cell current densities (pA/pF). The average cell capacitance was 41.3± 7.5 pF (n = 28 cells).

Drugs and chemicals

Papain, 4-aminopyridine (4-AP), nystatin, tetraethylammonium (TEA), sodium orthovanadate (VAN), genistein (GST) were purchased from Sigma. GST was prepared as a 50 mmol/L stock solution in DMSO and stored at -20 ℃. DTT (BBI), HEPES, DMSO, bovine serum albumin were from Shanghai Sangon Biological Engineering Technology and Services Company.

Statistical analysis

Data were expressed as mean±SD. Differences in the data were evaluated by paired or independent t-test when appropriate. $P<0.05$ was considered statistically significant. Software Microcal Origin 5.0 was used for statistical analysis and graph plotting.

RESULTS

Potassium currents in single smooth muscle cells

Outward potassium currents are mainly composed of voltage-gated potassium current and calcium-dependent potassium current. Under the conditions of our recordings (no added Ca^{2+} and 0.2 mmol/L $CdCl_2$ in the bath solution), contamination of currents through calcium-dependent potassium currents was minimized.

Currents were evoked using standard stimulus protocol, i.e., the membrane potential was stepped for 400 ms from a holding potential of -80 mV to test potentials between -80 and +50 mV in 10 mV increments. Depolarization to potentials positive to -40 mV activated non-linear, time-dependent outward currents which could be divided into transient outward potassium current (I_{Kto}) and delayed rectifier potassium current (I_{Kdr}) as shown in Figure 1. K_{to} was sensitive to millimolar concentration of 4-AP and insensitive to TEA. K_{dr} was on the contrary. When 10 mmol/L TEA was externally applied, early peak current of transient outward current (peak current among the first 50 ms of test pulse, I_{peak}) was only slightly decreased, but the quasi steady state current (average current from 350 to 400 ms after test pulse onset, I_{SS}) was blocked -60% (Figure 1A). When 5 mmol/L 4-AP was externally applied, I_{peak} was much reduced (Figure 1B) and the time to half-maximum current at test potential was significantly increased (data not shown), I_{SS} was little affected. Detailed description of voltage-dependent potassium current of guinea pig proximal colon smooth muscle cells could

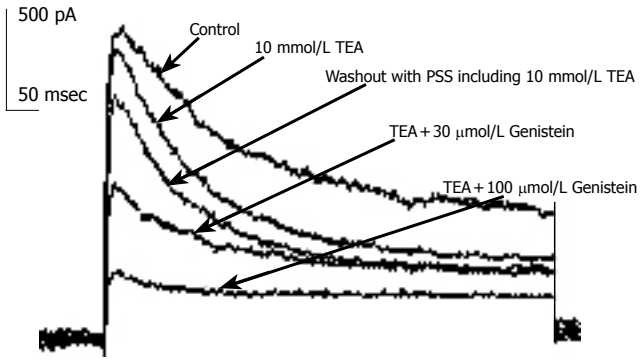

Figure 2 Concentration-dependent inhibition of transient outward potassium currents by genistein in guinea pig colon smooth muscle cells. Currents were elicited by depolarization to +50 mV for 400 ms then back to -40 mV from holding potential of -80 mV. Washout was performed for about 2 min.

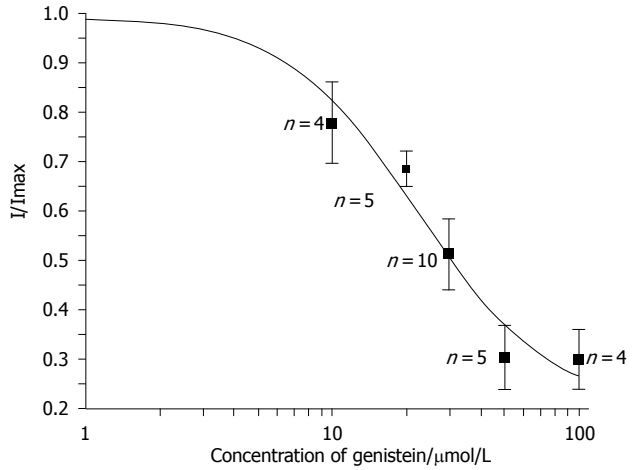

Figure 3 Average concentration-dependent transient outward potassium currents inhibited by genistein. Peak currents were measured and normalized by control current amplitude. The smooth line represents the best fitting with Hill equation. Data are expressed as mean±SE. The number of cells used in each concentration is indicated besides the error bars.

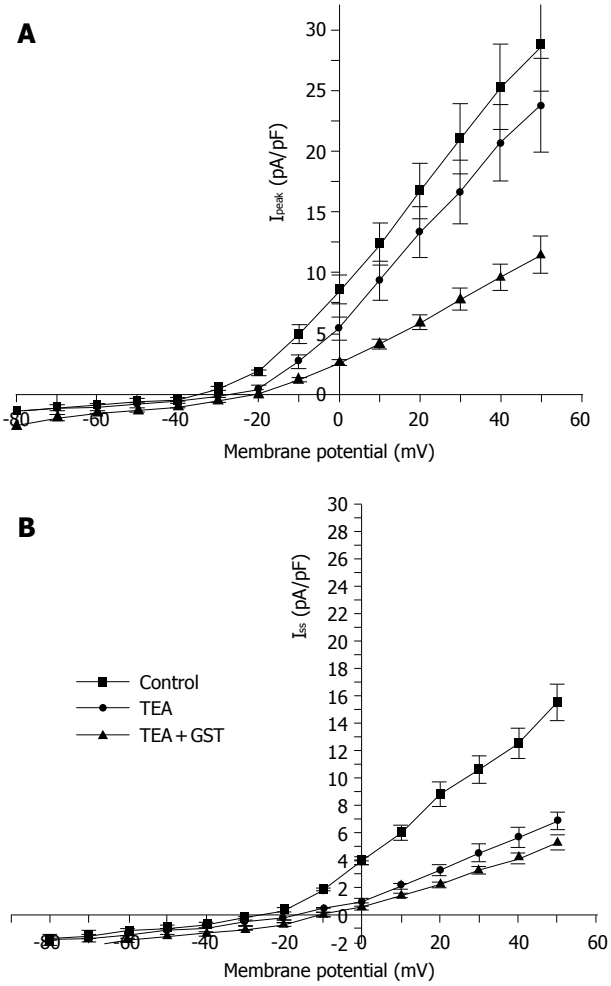

Figure 4 Effect of genistein (30 μmol/L) on current-voltage relationship of transient outward potassium current (I_{Kto}) in guinea pig proximal colon smooth muscle cells in the presence of TEA (10 mmol/L) and Cd^{2+} (0.2 mmol/L). **A**: *I-V* curves of peak current density of voltage-gated potassium channels under control condition (solid square), in the presence of 10 mmol/L TEA (solid circle) as well as 10 mmol/L TEA and 30 μmol/L genistein (solid triangle); **B**: *I-V* curves of steady state current density of voltage-gated potassium channels under control condition, in the presence of 10 mmol/L TEA as well as 10 mmol/L TEA and genistein (30 μmol/L). $n = 6$ cells.

be seen elsewhere[13], which agrees well with our study. According to the different sensitivity to TEA and 4-AP, we isolated these two kinds of current pharmacologically to study the effect of GST independently.

Concentration-dependent inhibition of GST on I_{Kto}

Currents recorded were mainly I_{Kto} when 10 mmol/L TEA was externally applied to bath solution. Repetitive single current traces were elicited for 400 ms from a holding potential of -80 mV to test potentials +50 mV in every 20s until stable currents were recorded. I_{peak} of K_{to} decreased progressively and reached a stable state in about 1-2 min after the external perfusion of GST. GST (10-100 μmol/L) induced a reduction in I_{peak}, with little effect on the steady state potassium current. The inhibitory effects of GST were reversible (Figure 2). Perfusion of calcium-free saline including TEA could restore the amplitude of currents to about 80% of control in 2 min (data not shown).

GST could not totally block the outward potassium currents even when 100 μmol/L GST was applied. Since no significant difference in the inhibitory effect was found between 50 ($n = 10$) and 100 ($n = 5$) μmol/L GST, a fraction of the outward potassium current was insensitive to GST.

Early peak transient outward potassium current of K_{to} measured at +50 mV test pulse from a holding potential of -80 mV was used as an index of inhibition, which was plotted as a function of GST concentration (10-100 μmol/L). The data were fitted with $I_{GST}/I_{control} = 1/\{1+(IC_{50}/[D])^n\}$, where [D] is the concentration of GST used, IC_{50} is the concentration at half maximal inhibition, and n is the Hill coefficient. Figure 3 shows that the effect of GST on I_{peak} of K_{to} was concentration-dependent, with an IC_{50} of 22.0±6.9 μmol/L.

Effect of GST on current-voltage relationship (I-V curves) of I_{Kto}

Currents were evoked using standard stimulus protocol. The membrane potential was stepped for 400 ms from a holding potential of -80 mV to test potentials between -80

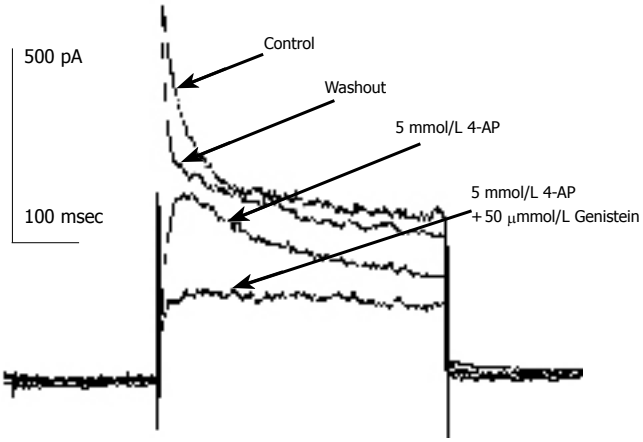

Figure 5 Effect of genistein on delayed rectifier potassium currents in the presence of 4-AP in guinea pig colon smooth muscle cells. Currents were elicited by depolarization to +50 mV for 400 ms then back to -40 mV from holding potential of -80 mV. Washout was performed for about 2 min.

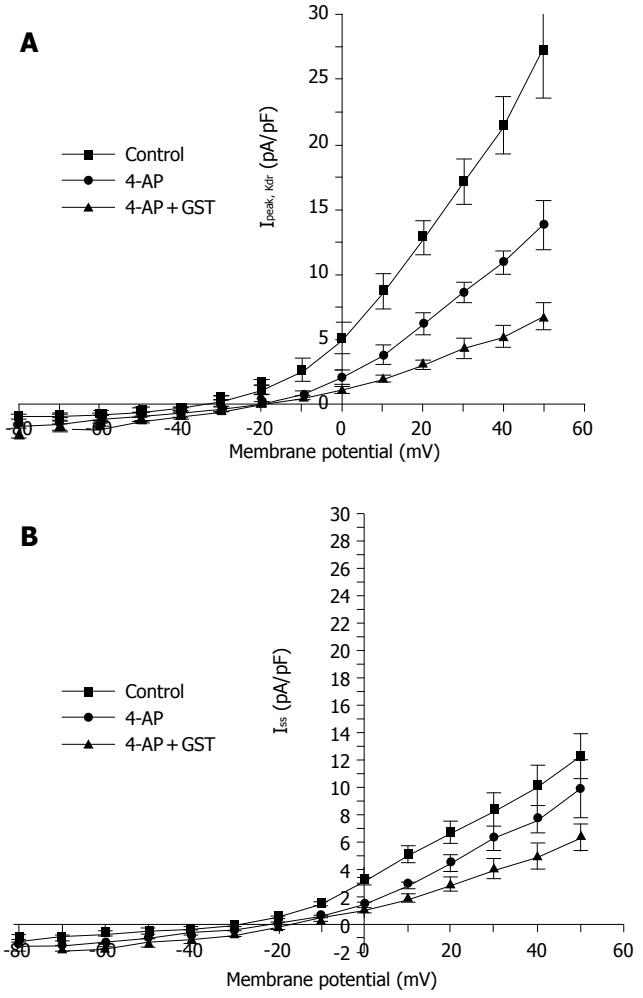

Figure 6 Effect of genistein (50 μmol/L) on current-voltage relationship of delayed rectifier potassium current (I_{Kdr}) in guinea pig proximal colon smooth muscle cells in the presence of 4-AP (5 mmol/L) and Cd^{2+} (0.2 mmol/L). **A**: *I-V* curves of peak current density of voltage-gated potassium channels under control condition, in the presence of 5 mmol/L 4-AP as well as 5 mmol/L 4-AP and 50 μmol/L genistein; **B**: *I-V* curves of steady state current density of voltage-gated potassium channels under control condition, in the presence of 5 mmol/L 4-AP as well as 5 mmol/L 4-AP and 50 μmol/L genistein. $n = 4$ cells.

and +50 mV in 10 mV increments. The interval between two pulses was set at 10 s to allow inactivated conductance to recover. I_{peak} and I_{SS} were measured at test pulses from -80 to +50 mV and converted into current densities, which were plotted as a function of test pulse.

Figure 1 shows that in the presence of 10 mmol/L TEA, 30 μmol/L GST significantly blocked I_{peak} ($P<0.05$, $n = 6$ cells) and only slightly reduced I_{SS}. Figure 4 shows the averaged *I-V* curves of I_{Kto} inhibited by GST. The inhibition showed no voltage dependence.

Effect of GST on I_{Kdr}

Currents recorded in the presence of external 5 mmol/L 4-AP were mainly delayed rectifier potassium currents. Repetitive single current traces were elicited for 400 ms from a holding potential of -80 mV to test potentials +50 mV in every 20 s until stable currents were recorded. In the presence of 5 mmol/L 4-AP, 50 μmol/L GST reduced peak current and steady state current with a fractional inhibition of current f [$f = (1-I_{GST}/I_{drug})\times 100\%$] of 52±8% ($P<0.05$, $n = 4$) and 33±2% ($P<0.05$, $n = 4$), respectively. The inhibitory effects of GST were reversible. Perfusion of calcium-free saline could restore the amplitude of currents in 2 min (data not shown). Details are shown in Figure 5.

Effect of GST on I-V curves of I_{Kdr}

The same experimental protocol as that of the study on the effect of GST on I_{Kto} was used. Figure 1B shows that in the presence of 5 mmol/L 4-AP, 50 μmol/L GST significantly blocked I_{peak} as well as I_{SS} ($P<0.05$, $n = 4$ cells). Figure 6 shows the averaged *I-V* curves of I_{Kdr} inhibited by GST. The inhibition showed no voltage dependence, and 50 μmol/L GST had a greater effect on I_{Kto} than on I_{Kdr} ($P<0.05$).

Effect of GST on steady state activation kinetics of I_{Kto}

The activation curves of I_{Kto} were derived from *I-V* curves of I_{Kto}. G_{Kto} was calculated by dividing the initial peak current value by their driving force and plotted as a function of membrane potential. The activation curves were fitted with a Boltzmann function $G/G_{max} = 1/\{1+\exp[(V-V_h)]/k\}$, where V is membrane potential, V_h is the half-maximal activation voltage, and k is the slope constant (mV). GST had no significant effect on steady state activation kinetics of I_{Kto}. The value of half-maximal activation voltage V_h was 3.3±1.3 and 3.5±1.4 mV, respectively in the absence and presence of 30 μmol/LGST, the slope constant k was 12.9±1.0 and 13.3±1.1 mV, respectively ($n = 6$, Figure 7).

Effect of GST on steady state inactivation kinetics of I_{Kto}

Classic double pulse protocol was used to determine the voltage dependence of inactivation of I_{Kto}. Currents were elicited by a depolarizing pulse of +50 mV with 1 s preconditioning pulses from -80 to 20 mV by increment of 10 mV. The interval between two sweeps was set at 20s. A plot of normalized peak current (I/I_{max}) as a function of preconditioning potential was fitted with a Boltzmann function: $I/I_{max} = 1/\{1+\exp[(V_h-V)]/k\}$, where V is the

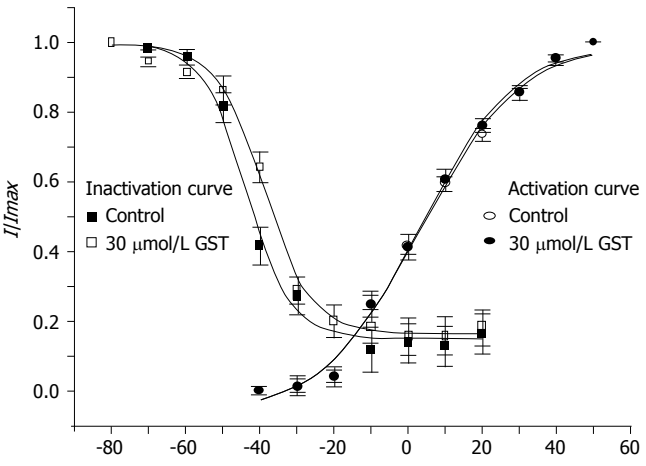

Figure 7 Effect of GST on steady state activation and inactivation of transient outward potassium currents. Inactivation is shown as a plot of normalized peak current as a function of conditional potential from -80 to +20 mV. For voltage dependence of activation, normalized currents were calculated by dividing peak outward currents by their driving force and plotted against potential. Smooth curves were fitted with Boltzmann equation which yields values of half-maximally activated or inactivated voltage (V_h) and slope constant (K).

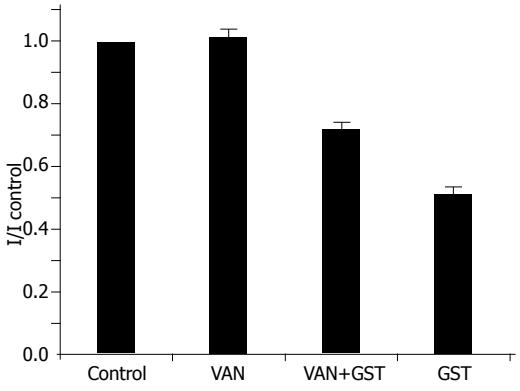

Figure 8 Orthovanadate antagonizes the inhibition of transient outward potassium currents by genistein.

membrane potential, V_h is the half-maximal inactivation voltage, and k is the slope constant (mV). GST had no significant effect on steady state inactivation kinetics of I_{Kto}. The values of half-maximal inactivation voltage V_h were -43.4±0.7 and -39.2±0.8 mV, respectively in the absence and presence of 30 μmol/L GST, the slope constant k was 5.8±0.6 and 6.5±0.7 mV, respectively (n=6, Figure 7).

VAN blocked inhibition of GST on I_{kto}

Inhibition of phosphatase-mediated tyrosine dephosphorylation would be expected to block current inhibition by GST, since dephosphorylation in the presence of GST would require ongoing phosphatase activity[14]. At commonly used bath concentrations of 0.1-10 mmol/L, inhibitor of tyrosine phosphatase orthovanadate could antagonize various GST-induced cellular responses such as blocking the inhibition of $I_{Ca,L}$ by PTK inhibitors[15]. Solely applied VAN had minor stimulating but no significant effect on I_{Kto} (n=7). In the presence of 1 mmol/L VAN, the fractional inhibition of current f of 30 μmol/LGST significantly decreased to 28±6% (Figure 1) compared with 49±6% of solely applied GST (n=10). The rapid onset and offset responses to GST did not change (Figure 8).

DISCUSSION

At least four types of potassium channel have been identified in excitable gastrointestinal smooth muscle cells, such as voltage-dependent outward potassium channel, calcium-dependent potassium channel, ATP-sensitive potassium channel, and inward rectifier potassium channel. A given smooth muscle cell can express several families of potassium channels and several members of a single family of channels. This complexity is necessary for the control of smooth muscle function[11]. Kv channels are of particular importance in the regulation of colonic smooth muscle electrical activity because they provide outward currents over the voltage range in which these tissues operate[11]. Kv shapes the action potential (AP) by controlling its repolarization phase and determines the membrane potential and duration of the interspike interval. Delayed rectifier potassium channels keep single AP short and permit high-frequency trains of APs. Transient outward potassium channels help a cell fire at low frequency and promote broadening of APs during repetitive activity[16]. In murine colon, application of 4-AP to intact preparations can abolish the quiescent periods between slow waves and induce a slight depolarization[11]. I_{Kto} is fully inactivated during the upstroke depolarization[17]. In this study, GST could block transient outward potassium currents to depolarize membrane potential as well as delayed rectifier potassium currents to induce slight depolarization[11], thus modulating the contraction of smooth muscle.

Protein tyrosine kinase activity is a major signaling mechanism in regulating long-term processes such as cell growth, division, and metabolism[18]. PTK signaling is also important in regulating ion channel conductance. GST, a natural isoflavone which is abundant in soybean, is a specific inhibitor of tyrosine specific kinases by competing with ATP to form the nonproductive enzyme-substrate complexes[19]. In this study, GST concentration - dependently and reversibly blocked voltage-gated potassium currents. Though it was reported that GST has no specific effects such as direct interaction with potassium channels[14,20,21], orthovanadate could antagonize the blockage of GST on I_{Kto}, suggesting that although direct blockage of potassium channels by a mechanism unrelated to PTK inhibition could not be entirely excluded in this study, GST blocks the transient outward potassium channels partly via PTK pathway.

There are some differences in GST action on ion channel currents and contraction of different types of smooth muscle. GST can inhibit visceral smooth muscle contraction induced by vanadate[8], angiotensin II[9] and carbachol[10] as well as nifidipine-sensitive calcium currents in rabbit colon myocytes[22]. GST also can inhibit apamin-sensitive relaxation of the longitudinal muscle in rat distal colon induced by pituitary adenylate cyclase activating peptide[23] as well as potassium channels[4,5]. Besides species and tissue differences, it is possible that multiple receptor

and nonreceptor PTKs are involved in the regulation of smooth muscle contraction. Further studies are required to identify the physiological role of PTKs in gastrointestinal motility.

To check whether GST could affect the biophysical kinetics of voltage-dependent potassium channels, we examined the steady state activation and inactivation kinetics in the absence and presence of 30 μmol/L GST. Peretz *et al*[4] found that GST affects potassium gating properties of Schwann cells such as a positive shift in voltage dependence of activation (by + 30 mV) and a decrease in steepness of activation gating. In our experiment, no significant effect of GST on gating properties of potassium channels was observed. Whether species or cell type difference contributes to the variation needs further investigation.

In conclusion, GST concentration dependently and reversibly inhibits transient outward potassium currents.

REFERENCES

1 **Davis MJ**, Wu X, Nurkiewicz TR, Kawasaki J, Gui P, Hill MA, Wilson E. Regulation of ion channels by protein tyrosine phosphorylation. *Am J Physiol Heart Circ Physiol* 2001; **281**: H1835-H1862

2 **Hopfield JF**, Tank DW, Greengard P, Huganir RL. Functional modulation of the nicotinic acetylcholine receptor by tyrosine phosphorylation. *Nature* 1988; **336**: 677-680

3 **Wang YT**, Salter MW. Regulation of NMDA receptors by tyrosine kinases and phosphatases. *Nature* 1994; **369**: 233-235

4 **Peretz A**, Sobko A, Attali B. Tyrosine kinases modulate K+ channel gating in mouse Schwann cells. *J Physiol* 1999; **519 Pt 2**: 373-384

5 **Holmes TC**, Fadool DA, Levitan IB. Tyrosine phosphorylation of the Kv1.3 potassium channel. *J Neurosci* 1996; **16**: 1581-1590

6 **Ji ES**, Yin JX, Ma HJ, He RR. Effect of genistein on L-type calcium current in guinea pig ventricular myocytes. *Shengli Xuebao* 2004; **56**: 466-470

7 **Strauss O**, Mergler S, Wiederholt M. Regulation of L-type calcium channels by protein tyrosine kinase and protein kinase C in cultured rat and human retinal pigment epithelial cells. *FASEB J* 1997; **11**: 859-867

8 **Alcón S**, Camello PJ, García LJ, Pozo MJ. Activation of tyrosine kinase pathway by vanadate in gallbladder smooth muscle. *Biochem Pharmacol* 2000; **59**: 1077-1089

9 **Yang SG**, Saifeddine M, Laniyonu A, Hollenberg MD. Distinct signal transduction pathways for angiotensin-II in guinea pig gastric smooth muscle: differential blockade by indomethacin and tyrosine kinase inhibitors. *J Pharmacol Exp Ther* 1993; **264**: 958-966

10 **Di Salvo J**, Steusloff A, Semenchuk L, Satoh S, Kolquist K, Pfitzer G. Tyrosine kinase inhibitors suppress agonist-induced contraction in smooth muscle. *Biochem Biophys Res Commun* 1993; **190**: 968-974

11 **Koh SD**, Ward SM, Dick GM, Epperson A, Bonner HP, Sanders KM, Horowitz B, Kenyon JL. Contribution of delayed rectifier potassium currents to the electrical activity of murine colonic smooth muscle. *J Physiol* 1999; **515 (Pt 2)**: 475-487

12 **Komori S**, Bolton TB. Role of G-proteins in muscarinic receptor inward and outward currents in rabbit jejunal smooth muscle. *J Physiol* 1990; **427**: 395-419

13 **Vogalis F**, Lang RJ, Bywater RA, Taylor GS. Voltage-gated ionic currents in smooth muscle cells of guinea pig proximal colon. *Am J Physiol* 1993; **264**: C527-C536

14 **Smirnov SV**, Aaronson PI. Inhibition of vascular smooth muscle cell K+ currents by tyrosine kinase inhibitors genistein and ST 638. *Circ Res* 1995; **76**: 310-316

15 **Ogura T**, Shuba LM, McDonald TF. L-type Ca2+ current in guinea pig ventricular myocytes treated with modulators of tyrosine phosphorylation. *Am J Physiol* 1999; **276**: H1724-H1733

16 **Oliver D**, Lien CC, Soom M, Baukrowitz T, Jonas P, Fakler B. Functional conversion between A-type and delayed rectifier K+ channels by membrane lipids. *Science* 2004; **304**: 265-270

17 **Amberg GC**, Baker SA, Koh SD, Hatton WJ, Murray KJ, Horowitz B, Sanders KM. Characterization of the A-type potassium current in murine gastric antrum. *J Physiol* 2002; **544**: 417-428

18 **Fantl WJ**, Johnson DE, Williams LT. Signalling by receptor tyrosine kinases. *Annu Rev Biochem* 1993; **62**: 453-481

19 **Akiyama T**, Ishida J, Nakagawa S, Ogawara H, Watanabe S, Itoh N, Shibuya M, Fukami Y. Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* 1987; **262**: 5592-5595

20 **Paillart C**, Carlier E, Guedin D, Dargent B, Couraud F. Direct block of voltage-sensitive sodium channels by genistein, a tyrosine kinase inhibitor. *J Pharmacol Exp Ther* 1997; **280**: 521-526

21 **Belevych AE**, Warrier S, Harvey RD. Genistein inhibits cardiac L-type Ca(2+) channel activity by a tyrosine kinase-independent mechanism. *Mol Pharmacol* 2002; **62**: 554-565

22 **Hatakeyama N**, Mukhopadhyay D, Goyal RK, Akbarali HI. Tyrosine kinase-dependent modulation of calcium entry in rabbit colonic muscularis mucosae. *Am J Physiol* 1996; **270**: C1780-C1789

23 **Takeuchi T**, Kishi M, Hirayama N, Yamaji M, Ishii T, Nishio H, Hata F, Takewaki T. Tyrosine kinase involvement in apamin-sensitive inhibitory responses of rat distal colon. *J Physiol* 1999; **514 (Pt 1)**: 177-188

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CLINICAL RESEARCH

Long-term outcome of endoscopic metallic stenting for benign biliary stenosis associated with chronic pancreatitis

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Abstract

AIM: Endoscopic metal stenting (EMS) offers good results in short to medium term follow-up for bile duct stenosis associated with chronic pancreatitis (CP); however, longer follow-up is needed to determine if EMS has the potential to become the treatment of first choice.

METHODS: EMS was performed in eight patients with severe common bile duct stenosis due to CP. After the resolution of cholestasis by endoscopic naso-biliary drainage three patients were subjected to EMS while, the other five underwent EMS following plastic tube stenting. The patients were followed up for more than 5 years through periodical laboratory tests and imaging techniques.

RESULTS: EMS was successfully performed in all the patients. Two patients died due to causes unrelated to the procedure: one with an acute myocardial infarction and the other with maxillary carcinoma at 2.8 and 5.5 years after EMS, respectively. One patient died with cholangitis because of EMS clogging 3.6 years after EMS. None of these three patients had showed symptoms of cholestasis during the follow-up period. Two patients developed choledocholithiasis and two suffered from duodenal ulcers due to dislodgement of the stent between 4.8 and 7.3 years after stenting; however, they were successfully treated endoscopically. Thus, five of eight patients are alive at present after a mean follow-up period of 7.4 years.

CONCLUSION: EMS is evidently one of the very promising treatment options for bile duct stenosis associated with CP, provided the patients are closely followed up; thus setting a system for their prompt management on emergency is desirable.

Key words: Chronic pancreatitis; Biliary stricture; Metallic stent; Long-term outcome

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INTRODUCTION

Chronic pancreatitis (CP) is reported to become complicated with bile duct stenosis in about 5-40% of the patients[1,2].The severity of the stenosis varies; however, radical treatment is needed for serious cases presenting persistent cholestasis, jaundice or cholangitis. Moreover, it has been shown that long-standing biliary stenosis, even if mild or moderate, often causes liver damage[3,4]. Half of these patients will present liver fibrosis by the time of decompression[3].

Non-surgical treatment has been reported to be comparable with surgery, with lower morbidity and mortality. Especially, endoscopic biliary drainage (ERBD) using a plastic tube stent has been adopted as the first-line treatment, and there are many reports concerning the usefulness of ERBD[5,6]. Although plastic stents offer satisfactory short-term drainage, medium to long-term results have been disappointing because of stent clogging or migration[6,7], and surgical treatment is chosen for a permanent palliation. However, surgical treatment is associated with important morbidity[8].

ERBD using a metallic stent (EMS) is useful for malignant biliary stricture since a relatively long-term non-surgical palliation can be attained[9]. In contrast, EMS for a benign biliary stricture is still controversial[10,11]. Furthermore, there have been few reports on EMS for biliary stenosis due to CP. Deviere *et al* reported favorable

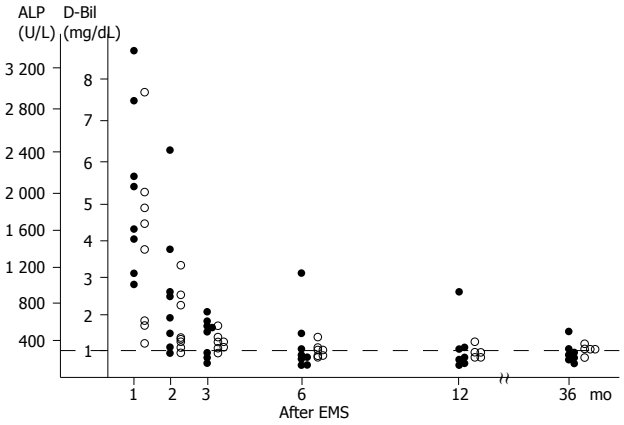

Figure 1 Plotting of the serum alkaline phosphatase concentration (●) and conjugated bilirubin (○) of each patient during 3 yr after EMS. The broken line indicates the upper limit of normal.

Table 1 Clinical background of the patients

Patient number	Age (yr)	Sex	Cause of CP	Underlying diseases	Jaundice	Cholangitis
1	78	Male	Alcohol	Angina Pectoris	+	+
2	53	Male	Alcohol	DM	-	+
3	42	Male	Alcohol	DM, Colon cancer	+	-
4	43	Male	Alcohol		-	-
5	77	Male	Alcohol		+	+
6	53	Male	Alcohol	DM	+	+
7	70	Male	Alcohol	DM	+	+
8	67	Male	Alcohol	DM	+	+

CP: Chronic pancreatitis; DM: Diabetes mellitus.

results with the self-expandable metal mesh stent for biliary obstruction due to CP, but the long-term results have not been reported[12]. In this study, we intended to clarify the outcome of patients with biliary stenosis due to CP who underwent EMS and have been followed up for more than 5 years.

MATERIALS AND METHODS

Between July 1996 and August 1998, EMS was performed in eight patients with severe common bile duct stenosis associated with CP in our institution. We had experienced 64 patients with CP during that period in the endoscopic retrograde cholangiopancreatography (ERCP) division. The indication for EMS was intractable bile duct stenosis uncontrollable by plastic tube biliary drainage. Ten patients gave their informed consent and the procedure was approved by the Ethical Committee of our institute; however, one patient underwent surgical treatment and another was lost to follow-up. They were all males with a median age of 65.7 years (range: 42-78 years). The etiology of CP was alcohol abuse in seven and idiopathic in one. Underlying diseases were diabetes mellitus in five patients, angina pectoris in one and early colon cancer in one. Mean duration of the illness before manifesting the first symptoms of bile duct stenosis was 3.7 years.

On their first admission, five patients presented with abdominal pain, six had symptomatic jaundice, and six had cholangitis (Table 1). Serum alkaline phosphatase concentration (ALP) was three times or more the upper limit of normal (mean 1 815 U/L; range 984-3 358) in all the patients. Similarly, serum conjugated bilirubin (D-Bil) was elevated above the upper limit of normal (mean 3.45 mg/dL; range 1.2-7.4) in seven patients. Abdominal ultrasonography and CT scan demonstrated marked dilatation of the common bile duct and slight to moderate dilatation of the intra-hepatic bile duct.

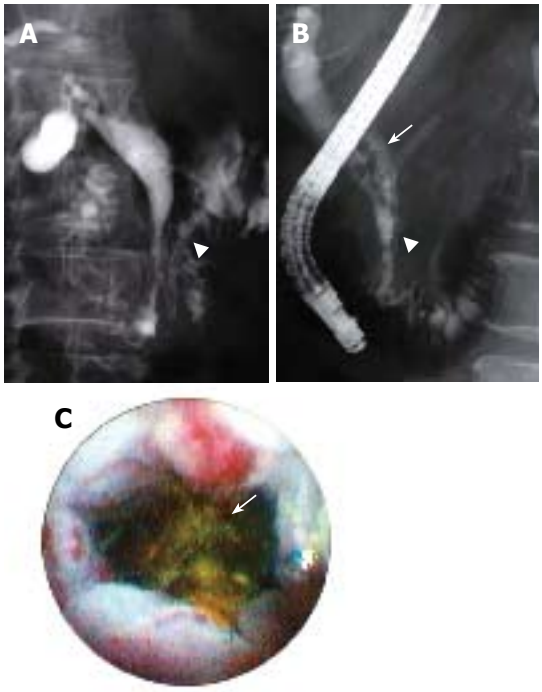

Figure 2 No bile duct stone was observed immediately after EMS in patient number 7 (**A**), however, stones were noted 6.1 years later (arrow), and they were successfully removed using a basket wire (**B**). Peroral cholangioscopy revealed the common bile duct distal to the stone (arrow) was patent and the hyperplastic change was not conspicuous (**C**). Arrow head indicates the pancreatic duct stent.

Endoscopic naso-biliary drainage (ENBD) using a 7-Fr. Plastic tube was performed in all the patients as the initial treatment. At that time, cholangiography showed almost complete obstruction or severe stenosis of the lower part of common bile duct. After the resolution of symptoms and laboratory findings of cholestasis by ENBD, three patients were subjected to EMS, while the other five underwent EMS after stenting using a 10 Fr. tube. The mean duration of tube stenting was 1.4 mo (range, 2-7 mo). Three patients developed clogging of the tube stent and subsequently the stent was changed by an EMS. In the other two patients, the tube stents were withdrawn; however, symptoms of re-obstruction were detected within 3 mo and thus EMS was performed.

For EMS, a non-covered, non-self-expandable stent (Strecker stent) was used in two patients and a self-expandable stent (Wallstent, Boston Scientific Corporation, Natick, MA, USA) was used in the other six patients, the length and internal diameter of the stents were 4 and 5 cm, 7 and 10 cm after full expansion, respectively.

In four patients, plastic tube stents were inserted to resolve the main pancreatic duct stenosis, and they were

Table 2 Clinical course of the patients after EMS

Patient number	Cholestasis	Cholangitis	Stone formation	Dislodgement	Alcohol intake	Outcome	Duration of stent patency(yr)
1	+ 1 mo	+ 1 mo	-	-	-	Dead	2.8
2	+ 4.6 yr	-	-	-	-	Alive	8.3
3	+	-	+ 7.3 yr	+ 5.9 yr	+	Alive	7.3
4	-	-	-	-	-	Alive	7.1
5	-	-	-	+ 4.8 yr	-	Alive	6.9
6	-	-	-	-	-	Dead	5.5
7	+	-	+ 6.1 yr	-	+	Alive	6.1
8	+ 3.6 yr	+ 3.6 yr	-	-	+	Dead	3.6

occasionally inserted depending on the patient's symptoms: abdominal and/or back pain.

After EMS, periodical examination of hemogram, blood biochemistry, urinalysis, and abdominal ultrasonography were conducted every 3-4 mo. Examination by cholangiography was occasionally performed, when a patient presented symptoms of biliary obstruction.

RESULTS

Short and medium-term (up to 3 years) follow-up after EMS (Table 2)

EMS was successfully performed in all the patients; however, in one patient (no. 1) clogging was observed within 1 mo and another EMS was inserted inside the first EMS. The patient showed no symptoms of bile duct obstruction thereafter.

The serum concentration of ALP and D-bil markedly decreased after the initial treatment including EMS (Figure 1), and 1 year after EMS, five of eight patients showed normal values, two patients showed slightly higher than the upper limit of normal, and one patient had a high serum concentration of ALP. Two years after EMS, none of the patients showed symptoms of bile duct obstruction and abdominal US demonstrated no dilatation of the intrahepatic bile duct with the stent located at the original position.

One patient (no. 1) died of acute myocardial infarction 2.8 years after EMS without symptoms of cholestasis during the follow-up period. The course of the other seven patients was uneventful for 3 years.

Long-term follow-up after EMS (Table 2)

One patient (no. 8) died of cholangitis (acute obstructive suppurative cholangitis, AOSC) caused by EMS clogging 3.6 years after EMS. The patient had presented no symptoms up to 1 wk before the last admission, when he felt acute pain in the right upper abdomen and experienced a sudden rise of his body temperature. He was brought to the hospital in a state of shock and was admitted to the ICU, but intensive care including ENBD was not effective and he died 17 d after the last admission. The patient's periodical check up with US and laboratory tests showed no signs of marked cholestasis except slight elevation of serum ALP concentration during the 3.5 years of follow-up as well as 1 mo before. It was suggested from the medical chart that the patient had suffered from cholangitis caused by stent clogging 1 wk before his admission, but he did not take it seriously; he thought that the symptoms were those of common cold, and delayed his visit to the hospital. Another patient (no. 2) presented

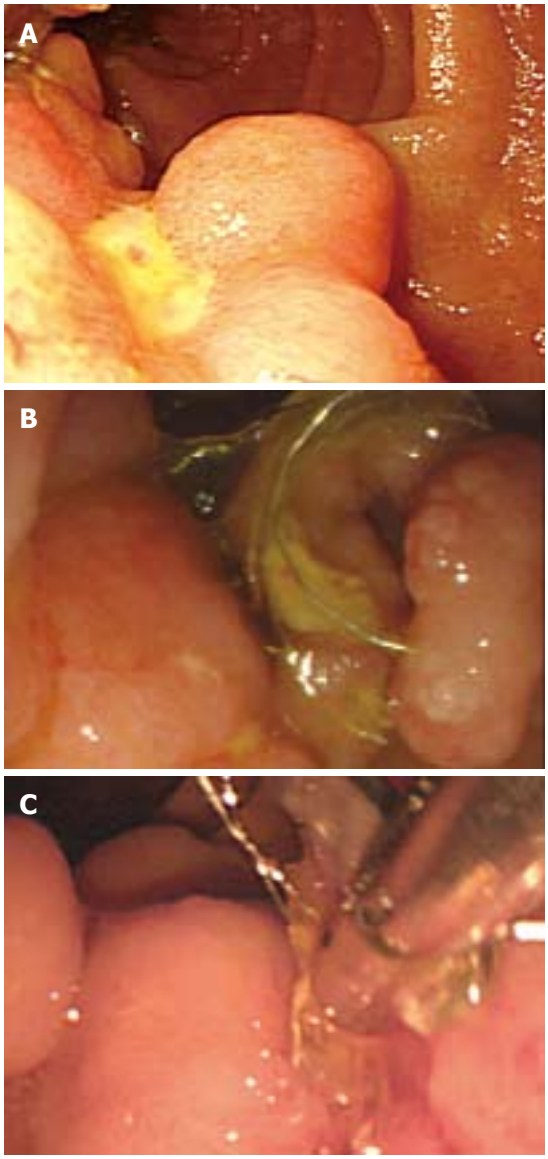

Figure 3 Endoscopic view showing a duodenal ulcer on the side opposite to the duodenal papilla (**A**) 5.9 years after the EMS in patient number 3. The stent had become dislodged and the steel wires protruded from the papilla (**B**). They were efficiently cut using the end-cutter (arrow) (**C**).

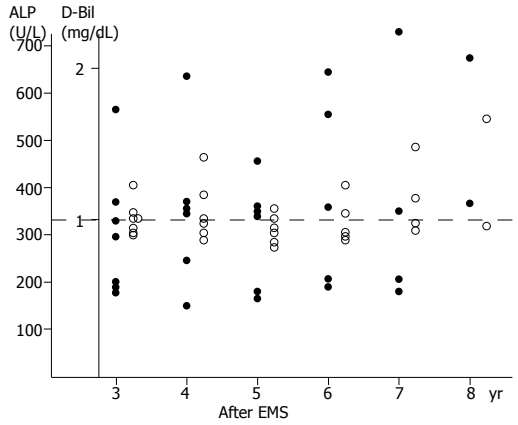

Figure 4 Plotting of the serum alkaline phosphatase concentration (●) and conjugated bilirubin (○) of each patient 3 yr after EMS. The broken line indicates the upper limit of normal.

with signs of cholestasis caused by acute exacerbation of pancreatitis 4.6 years after EMS; however, his condition improved soon after the treatment for pancreatitis without any endoscopic treatment, and he showed no symptoms thereafter. Two other patients (nos. 3 and 7) presented with cholestasis caused by choledocholithiasis in the upper part of the CBD proximal to the stent 7.3 and 6.1 years after EMS, respectively. In one patient (no. 7) the stones were successfully removed with a basket wire and he presented no symptoms thereafter (Figures 2A and 2B). In the other patient (no. 3) the stone could not be removed with a basket wire and two sessions of endoscopic hydraulic lithotripsy were necessary to eliminate it. Cholangioscopic examination of these patients showed that the metallic mesh was embedded into the bile duct wall allowing sufficient inner space without epithelial hyperplasia within the stent (Figure 2C).

Two patients (nos. 3 and 5) suffered from duodenal ulcers at 5.9 and 4.8 years after EMS, respectively, because the EMS wire dislodged and protruded from the papillary orifice, contacting the duodenal wall on the opposite side. The wire of the protruding part was cut under endoscopy with the end-cutter (Olympus, Tokyo, Japan), and the ulcer healed later (Figure 3).

Patient (no. 6) died of maxillary carcinoma 5.5 years after EMS although he presented no symptoms of cholestasis during the follow-up period.

Thus, five of the eight patients are alive at present and their mean follow-up period is already 7.4 (range: 6.5-8.3 years) years. The value of ALP is over the upper limit of normal in three patients now (Figure 4), and mild dilatation of the intra-hepatic bile duct is observed in three of them.

Patients (nos. 3, 6, 7, and 8) continued drinking alcohol after EMS; two of them developed choledocholithiasis and one suffered from AOSC. The other four patients did not drink alcohol after EMS and only one showed mild, transient signs of cholestasis.

DISCUSSION

EMS for benign bile duct stenosis as a complication of CP has been attempted in some institutes and patency of the stent is likely to be better[12-16]. Kahl *et al* used metal stents as the permanent treatment option and their patients remained free of obstructive jaundice or cholangitis for 1 year[16]. Deviere *et al* conducted a prospective study with a mean follow-up of 33 mo, and the stent lumen remained patent and functional throughout the follow-up period[12]. The reported results agree with our study concerning short to medium-term follow-up after EMS. In fact within 3 years after the treatment, all the patients except one who died of a heart attack showed almost uneventful clinical courses regarding biliary stenosis; judging from symptoms, laboratory findings including ALP, D-Bil, and imaging findings. One elderly patient died of causes other than CP with no signs of bile duct re-obstruction for 2.8 years. Such a good outcome was not achieved with the plastic tube stent[5,6,8,9,17], and long-term outcome of treatment with multiple tube stents was also disappointing[18].

Based on these results, EMS is obviously better than tube stenting regarding patency and not requiring scheduled changing of the stent. These advantages are thought to come from the characteristics of EMS: large caliber and expanding effect. However, the main problem of EMS is the development of epithelial hyperplasia in the stent resulting in obstruction[18]. Deviere *et al* reported that only two of 20 patients developed epithelial hyperplasia leading to cholestasis and jaundice within six months after stenting[12]. One of our patients also developed early obstruction of EMS, but an additional stent resolved this difficult problem. The metallic mesh is thought to become embedded in the bile duct wall early after stenting, so that a continuous membrane covers the inner stent[12]. Based on this hypothesis, ingrowth within the stent and subsequent obstruction may be related to the tumor rather than to CP, and in fact, the majority of our patients did not develop an early obstruction.

Although good results have been reported after a short- to medium-term follow-up, long-term results are unknown. Hastier *et al* reported the outcome of a patient treated with a metallic stent, who presented with no evidence of recurrence of cholestasis or episodes of cholangitis for a relatively long period of 3 years[14]. Van Berkel *et al* recently reported that they treated 13 patients with biliary stricture due to CP by self-expanding metal stent (SEMS) with the mean follow-up for 50 mo and that SEMS was found to be safe and provides successful as well as prolonged drainage in selected patients[19]. Nevertheless, the opinions in our institute were controversial regarding uncertainty of safety and outcome of EMS in patients with CP after a long-term follow-up, and we voluntarily decided to stop the application of EMS treatment from August 1998 until December 2003, when we could assess the long-term outcome and re-started EMS. Since then, two patients have been subjected to EMS up to now.

The outcome of EMS after more than 3 years was fairly good. But we experienced a very serious complication caused by re-obstruction of the bile duct. Emergency treatment was delayed leading to disappointing results in this case. If adequate intensive treatment had been started much earlier, the patient would have been saved. Another patient showed mild cholestatic symptom due to acute exacerbation of pancreatitis, but recovered soon after pancreatitis resolved without any intervention,

suggesting cholestasis was not caused by clogging of the stent but by another factor such as papillary edema. In two other patients with stone formation, cholangioscopic findings showed the patency of the stent lumen, according to the assumption of Deviere *et al*[12]. However, stones were thought to develop as a consequence of stagnation of bile, accordingly complete bile flow may be difficult to obtain even with EMS. Another complication of dislodgement was duodenal ulcers which were well controlled by endoscopic treatment.

One of our patients died of maxillary cancer long after EMS. Hastier *et al* decided to insert a metallic stent in their patient in view of his poor prognosis associated with the pulmonary malignancy[14]. CP is shown to frequently associate with various malignancies and a significantly lower survival rate compared with non-CP[20-22]. Therefore, in choosing EMS, this is one of the decision-making facts as well as the patients' general conditions and the lack of response to stenting using a plastic tube[2,13].

It is difficult to determine the optimal indications for EMS from our results because of the limited number of patients and the absence of a control group. However, since five of the eight patients are alive and leaving ordinary lives 7.4 years after EMS, this procedure is evidently one of the very promising options of treatment for bile duct stenosis in patients with CP, provided they are closely followed up through periodical check-ups. Thus setting a system for prompt management in case of an emergency is desirable. In addition, our results indicate that alcohol intake may be related to the poorer prognosis of patients subjected to EMS; thus prohibition of alcohol consumption is essential.

REFERENCES

1 **Wilson C**, Auld CD, Schlinkert R, Hasan AH, Imrie CW, MacSween RN, Carter DC. Hepatobiliary complications in chronic pancreatitis. *Gut* 1989; **30**: 520-527
2 **Schutz SM**, Baillie J. Another treatment option for biliary strictures from chronic pancreatitis. *Am J Gastroenterol* 1995; **90**: 1023-1024
3 **Lesur G**, Levy P, Flejou JF, Belghiti J, Fekete F, Bernades P. Factors predictive of liver histopathological appearance in chronic alcoholic pancreatitis with common bile duct stenosis and increased serum alkaline phosphatase. *Hepatology* 1993; **18**: 1078-1081
4 **Hammel P**, Couvelard A, O'Toole D, Ratouis A, Sauvanet A, Fléjou JF, Degott C, Belghiti J, Bernades P, Valla D, Ruszniewski P, Lévy P. Regression of liver fibrosis after biliary drainage in patients with chronic pancreatitis and stenosis of the common bile duct. *N Engl J Med* 2001; **344**: 418-423
5 **Devière J**, Devaere S, Baize M, Cremer M. Endoscopic biliary drainage in chronic pancreatitis. *Gastrointest Endosc* 1990; **36**: 96-100
6 **Barthet M**, Bernard JP, Duval JL, Affriat C, Sahel J. Biliary stenting in benign biliary stenosis complicating chronic calcifying pancreatitis. *Endoscopy* 1994; **26**: 569-572
7 **Godil A**, Chen YK. Endoscopic management of benign pancreatic disease. *Pancreas* 2000; **20**: 1-13
8 **Smits ME**, Rauws EA, van Gulik TM, Gouma DJ, Tytgat GN, Huibregtse K. Long-term results of endoscopic stenting and surgical drainage for biliary stricture due to chronic pancreatitis. *Br J Surg* 1996; **83**: 764-768
9 **Menon K**, Romagnuolo J, Barkun AN. Expandable metal biliary stenting in patients with recurrent premature polyethylene stent occlusion. *Am J Gastroenterol* 2001; **96**: 1435-1440
10 **Yoon HK**, Sung KB, Song HY, Kang SG, Kim MH, Lee SG, Lee SK, Auh YH. Benign biliary strictures associated with recurrent pyogenic cholangitis: treatment with expandable metallic stents. *AJR Am J Roentgenol* 1997; **169**: 1523-1527
11 **Lopez RR**, Cosenza CA, Lois J, Hoffman AL, Sher LS, Noguchi H, Pan SH, McMonigle M. Long-term results of metallic stents for benign biliary strictures. *Arch Surg* 2001; **136**: 664-669
12 **Deviere J**, Cremer M, Baize M, Love J, Sugai B, Vandermeeren A. Management of common bile duct stricture caused by chronic pancreatitis with metal mesh self expandable stents. *Gut* 1994; **35**: 122-126
13 **Kahl S**, Zimmermann S, Genz I, Glasbrenner B, Pross M, Schulz HU, Mc Namara D, Schmidt U, Malfertheiner P. Risk factors for failure of endoscopic stenting of biliary strictures in chronic pancreatitis: a prospective follow-up study. *Am J Gastroenterol* 2003; **98**: 2448-2453
14 **Hastier P**, Buckley JM, Peten EP, Dumas R, Delmont J. Long term treatment of biliary stricture due to chronic pancreatitis with a metallic stent. *Am J Gastroenterol* 1999; **94**: 1947-1948
15 **Van Westerloo DJ**, Bruno MJ, Bergman JJ, Cahen DL, van Berkel AM. Self-expandable wallstents for distal common bile duct strictures in chronic pancreatitis. Follow-up and clinical outcome of 15 patients. *Gastroenterology* 2000; **118**: A913
16 **Kahl S**, Zimmermann S, Glasbrenner B, Pross M, Schulz HU, McNamara D, Malfertheiner P. Treatment of benign biliary strictures in chronic pancreatitis by self-expandable metal stents. *Dig Dis* 2002; **20**: 199-203
17 **Farnbacher MJ**, Rabenstein T, Ell C, Hahn EG, Schneider HT. Is endoscopic drainage of common bile duct stenoses in chronic pancreatitis up-to-date? *Am J Gastroenterol* 2000; **95**: 1466-1471
18 **Mastrović Z**. [Ankylosing spondylitis as a social and medical problem]. *Reumatizam* 1975; **22**: 169-176
19 **Cahen DL**, van Berkel AM, Oskam D, Rauws EA, Weverling GJ, Huibregtse K, Bruno MJ. Long-term results of endoscopic drainage of common bile duct strictures in chronic pancreatitis. *Eur J Gastroenterol Hepatol* 2005; **17**: 103-108
20 **Lowenfels AB**, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, DiMagno EP, Andrén-Sandberg A, Domellöf L, Di Francesco V. Prognosis of chronic pancreatitis: an international multicenter study. International Pancreatitis Study Group. *Am J Gastroenterol* 1994; **89**: 1467-1471
21 **Lankisch PG**. Natural course of chronic pancreatitis. *Pancreatology* 2001; **1**: 3-14
22 **Malka D**, Hammel P, Maire F, Rufat P, Madeira I, Pessione F, Lévy P, Ruszniewski P. Risk of pancreatic adenocarcinoma in chronic pancreatitis. *Gut* 2002; **51**: 849-852

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Favorable outcomes of hilar duct oriented hepatic resection for high grade Tsunoda type hepatolithiasis

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Abstract

AIM: To evaluate the efficacy of hilar duct oriented hepatectomy for intractable hepatolithiasis, the ventral hilum exposure (VHE) method that has been applied by the authors.

METHODS: From June 1994 to June 2004 for a period of 10 years, 153 patients who had Tsunoda type III or IV hepatolithiasis, received hepatectomy at our institution. Among these patients, 128 who underwent hepatectomy by the VHE method were the subjects for the study. We analyzed the risk of this procedure, residual rate of intra-hepatic stones, and stone recurrent rates.

RESULTS: The average age was 54.2 years, and the male to female ratio was 1:1.7. The average follow-up period was 25.6 mo (6-114 mo). There was no post-operative severe complication or mortality after the operation. The rate of residual stones was 5.4% and the rate of recurrent stones was 4.2%.

CONCLUSION: VHE is a safe surgical procedure and provides favorable treatment results of intractable hepatolithiasis. Especially, this procedure has advantage in that intra-hepatic bile duct stricture may be confirmed and corrected directly during surgery.

Key words: Hepatolithiasis; Hepatic resection; Residual stone; Recurrent stone

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INTRODUCTION

Although hepatolithiasis is a benign condition, the clinical progression of this disease not only often limits a patient's social activities, sometimes to an extreme degree, but also may lead to liver parenchymal destruction due to recurrent cholangitis or even septic death if adequate treatment is not provided. Moreover, cholangiocarcinoma is found in approximately 5-10% of patients with hepatolithiasis[1,2]. The incidence of hepatolithiasis in the Korean population has been reported as 15% of all biliary tract stones, which is relatively higher than the data reported in Western populations[3].

In clinical practice, however, the treatment results are less favorable when compared to those of extrahepatic bile duct stones. The reason for this is the stricture of the intra-hepatic duct as a major cause of hepatolithiasis[4,5], which is commonly intractable because of the anatomical characteristics. It has been known that the most important aspects in the treatment of hepatolithiasis is the complete removal of all intra-hepatic stones and resolution of accompanied intra-hepatic duct stricture, in order to reduce the incidence of post-operative cholangitis and stone recurrence[6].

Recent developments in endoscopic techniques have also been shown to result in favorable outcomes[7]. Such advances have led to the shifting of surgical practice from uniform surgery to a combination of surgery and endoscopy for the treatment of hepatolithiasis[8,9]. But the endoscopic approach for hepatolithiasis is not always successful due to various intra-hepatic duct anatomies and cannot solve concomitant liver atrophy or latent cholangiocarcinoma.

The authors of this study implemented a surgical approach for hepatolithiasis according to the Tsunoda classification of hepatolithiasis, which describes the intra-hepatic duct in terms of dilatation and strictures, and is as follows: type I, no marked dilatation or strictures of intra-hepatic bile ducts; type II, diffuse dilatation of the intra-hepatic biliary tree without intra-hepatic duct strictures and frequently a stricture of the distal common bile duct; type III, unilateral solitary or multiple cystic intra-hepatic dilatation, frequently accompanied by stenosis of the left or right intra-hepatic bile ducts; and type IV, the same attributes as type III but with bilateral involvement of hepatic lobes[10]. The authors' policy of treatments in hepatolithiasis is according to Tsunoda type, that is, the type I or II is treated with endoscopic approach and the type III or IV is with surgical approach.

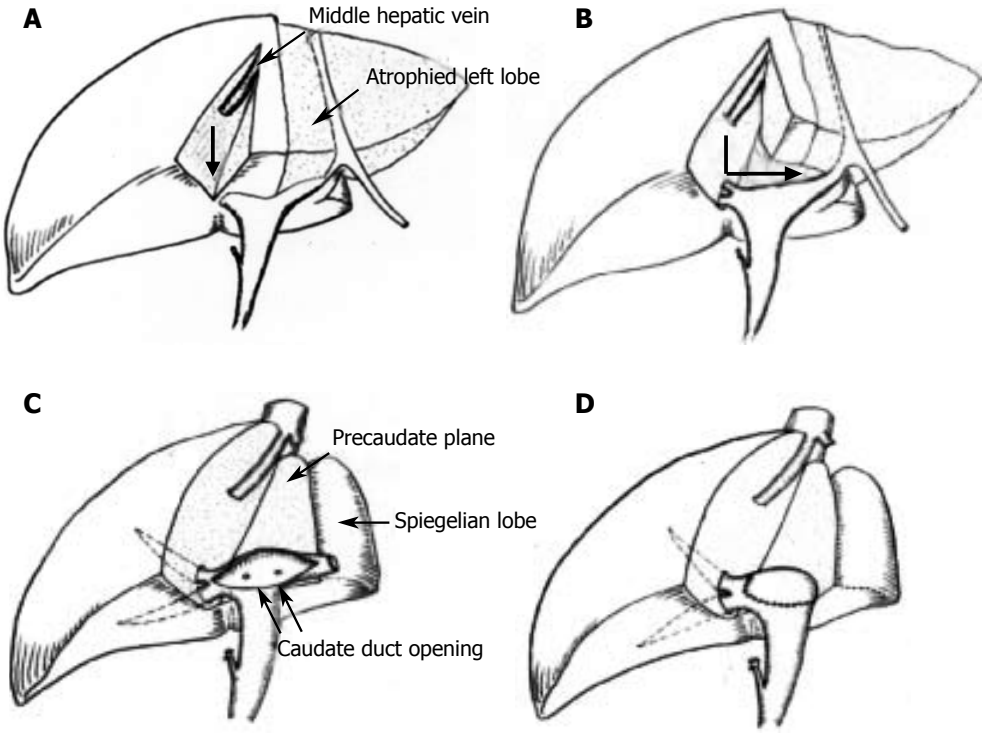

Figure 1 Schematic drawing of VHE procedure during left lobectomy. **A:** Hepatotomy commences vertically from the Cantlie's line to the direction of the hilar bile duct (thick arrow); **B:** after reaching the hilum, the hepatotomy is continued along the pre-caudate plane (angled thick arrow); **C:** the ventral portion of the hilar hepatic duct is opened along its direction; **D:** after completion of the necessary ductal procedure.

MATERIALS AND METHODS

From June 1994 to June 2004, a retrospective review was undertaken of 153 patients who received partial hepatectomy for hepatolithiasis at our institution, among whom, 128 patients underwent hilar duct oriented hepatectomy (ventral hilum exposure method, VHE). We analyzed the surgical risk of VHE method, residual rate of intra-hepatic stones, stone recurrent rates, and those patients with concomitant cholangiocarcinoma were also investigated.

The definition of hepatolithiasis was according to the description by Couinaud in which a gallstone is present in the proximal portion of the confluence of the common hepatic duct. The presence of residual or recurrent hepatolithiasis after surgery were assessed by ultrasonography (US), computerized tomography (CT) scans, cholangiograms by endoscopic retrograde cholangiopancreaticography (ERCP) and/or percutaneous transhepatic cholangiography (PTC). The postoperative follow-up comprised of US every 3 mo and CT scan every 6 mo for the first three years, and then once a year thereafter, or when abnormal symptoms suspicious of cholangitis or residual or recurrent hepatolithiasis were present. If the residual or recurrent stones were suspected by US or CT scan, the PTC and/or ERCP were introduced.

Recurrent hepatolithiasis was defined as stones recurring after 2 years of initial therapy. Operative mortality and hospital mortality was defined as death of a patient within 30 d of surgery, and death before discharge from the hospital after surgery, respectively.

Operative techniques

The goal of VHE is total exposure of the hilar bile duct. In case of left lobectomy, first the course of middle hepatic vein and the distribution of intra-hepatic stones are confirmed by intra-operative US, then the gallbladder is removed. A sagittal liver parenchyma dissection (hepatotomy) along the Cantlie's line or midline of the GB bed is done until reaching the right hilar Glisson's sheath. Then the sagittal dissection plane is rotated 90° to the left along the coronal plane or pre-caudate plane[11]. The preservation of the middle hepatic vein during sagittal hepatotomy is optional. Managed thus, long segment of the ventral portion of hilar Glisson's sheath can be separated from dorsal part of the segment IVb liver parenchyma (Figures 1A and 1B). Without exception in our experience, the hepatic hilar bile duct is situated at the extreme antero-superior aspect in the hilar Glisson's pedicle that can be confirmed by needle puncture aspiration of bile, and then the anterior wall of the hilar bile duct can be opened safely along its direction without the need of dissection of hilar Glisson's sheath (Figures 1C and 1D).

In this way, direct visualization of the openings of the second order branches of the intra-hepatic bile duct allows pronounced accuracy of intra-operative cholangioscopy in each of the intra-hepatic duct branches, and precise evaluation of the distribution of intra-hepatic stones and hilar stricture, and plastic reconstruction of the strictured hilar hepatic duct. Plastic reconstruction of intra-hepatic duct results in avoidance of a hepatico-jejunostomy in almost every patient, and also primary closure of the bile duct incision site maintains the physiologic anatomy of the intra-hepatic bile duct. The VHE procedure also resulted in easier confirmation of the common bile duct status, and none of the patients who received this therapy required a T-tube choledochostomy (Figures 2 and 3).

We did not perform VHE in cases where the hepatolithiasis was situated in the third branch or the more periphery of the intra-hepatic bile duct, when parenchymal destruction of the liver was observed in the periphery, and when no stricture was present up to the second

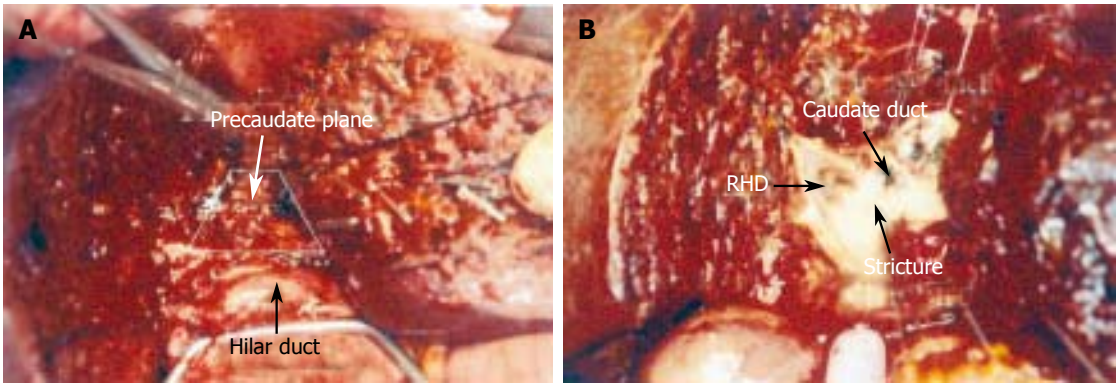

Figure 2 A: Dissecting along the pre-caudate plane allows the ventral portion of the hilar duct to be exposed during left lobectomy; **B:** the hilar duct was opened along its direction; RHD: right hepatic duct opening.

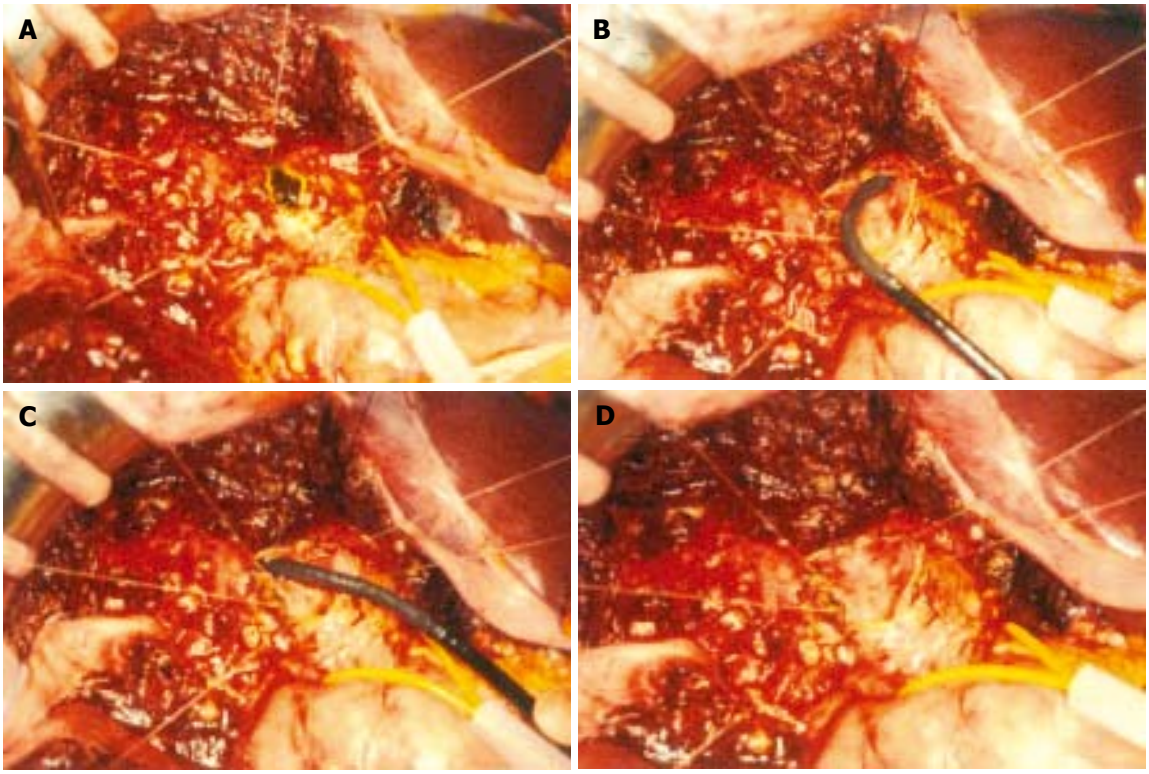

Figure 3 During central lobectomy, the hilar duct was exposed and opened. **A:** Extraction of the IHD stones; **B** and **C:** application of intra-operative choledochoscope to the left and right IHD; **D:** after the removal of all intra-hepatic stones.

order branches of the hilar bile duct. These patients were managed with conventional liver parenchyma resection (Table 1).

RESULTS

The average age of patients who received VHE surgery was 54.2 years, and the male to female ratio was 1:1.7. All 128 patients who underwent VHE surgery were Tsunoda type III or IV (98 patients were Tsunoda type III, and 30 were type IV). The type of stone in all the 153 patients who received surgery was pigment stone, and hilar duct stricture was confirmed in 90 of 128 patients who had VHE surgery (70%). In the remaining 38 patients without hilar ductal stricture, strictures were observed in the second order branches.

The average follow-up period of 128 patients after VHE for hepatolithiasis was 25.6 months (range; 6-114 mo). The number of patients with residual stones after VHE hepatectomy was 7/128 (5.4%), and residual stones were found in 4/98 patients with Tsunoda type III (4.1%) and in 3/30 with type IV (10%). Among them, there were six patients in whom residual stone removal by PTCS was performed who had symptoms such as cholangitis. And the remaining four asymptomatic patients were being followed up only with observation to date.

We could follow-up 48 patients with CT scan and US for more than two years after complete stone removal by the VHE method. The range of the follow-up period of these 48 patients was 26-116 mo, with an average of

Table 1 Type of hepatic resection

Type of operation	*n*	
Explo-laparotomy only[1]	6	Non-VHE (25)
LLS[2]	14	
LLS+RPS[3]	2	
RPS	3	
CL[4]	4	VHE (128)
LL[5]±subsegmentectomy	114	
RL+IVb[6]	10	
Total	153	

[1]Explo-laparotomy only due to carcinomatosis of cholangiocarcinoma. [2]Left lateral segmentectomy. [3]Right posterior segmentectomy. [4]Central lobectomy. [5]Left lobectomy. [6]Right lobectomy+subsegmentectomy IVb.

Table 2 Rate of residual and recurrent stones after VHE procedure (*n* = 128) according to Tsunoda type

Tsunoda type	Number of residual stone (%)	Number of recurrent stone (%)
III	4/98 (4.1)	2/39 (5.1)
IV	3/30 (10)	0/9 (0)
Total	7/128 (5.4)	2/48 (4.2)

60.4 mo. Among them, only two patients had recurrent stones, showing a recurrence rate of 4.2% (2/48). Both of these patients were Tsunoda type III at the time of surgery (Table 2). And these two recurrent patients developed cholangitis, and one died of sepsis as a result of the cholangitis. Among the 46 patients who were followed up for more than 2 years without recurrent stones, 2 developed acalculous cholangitis as the complication and were managed by conservative treatment. One patient was diagnosed with cholangiocarcinoma 3 years after surgery without evidence of stone recurrence (Table 3).

Among the total of 153 patients who received hepatectomy for hepatolithiasis, there were 17 cases of cholangiocarcinoma (11%), and six of these patients were of advanced state of unresectable disease during laparotomy. There were also three patients with *in situ* cholangiocarcinoma after confirmation of pathology. And only three patients were pre-operatively determined to be with cholangio-carcinoma among the total of 17 cases of concomitant cholangiocarcinoma.

There was no case of operative mortality or hospital mortality in all patients who were studied. Post-VHE complications occurred in 33/128 patients (25.7%), and consisted of wound infections (20 patients, the most common complication), pulmonary complications such as pleural effusion or pneumonia (nine patients), bile leakage (four patients), and one case each of post-operative minor intra-abdominal hemorrhage, intraperitoneal abscess, and gastrointestinal bleeding. All of the complicated cases were successfully managed conservatively.

Table 3 Long-term results in patients (*n* = 48) who underwent complete stone removal by VHE

Long-term results	Number of cases (%)
Free of symptoms and no recurrent stones	43 (89.6)
Recurrent stones with cholangitis	2 (4.2)
Cholangitis without recurrent stones	2 (4.2)
Cholangiocarcinoma	1 (2.1)

DISCUSSION

Recent therapy by the combination of endoscopy and surgery for the treatment of hepatolithiasis has allowed overcoming the limitations of each modality and therefore has led to marked enhancement of treatment results. Another reason for the increased treatment success may be due to the more accurate pre-operative evaluation of intra-hepatic bile duct strictures and distribution of intra-hepatic stones.

Many favorable results have been reported in the literature with regard to endoscopy in the management of hepatolithiasis[7,12]. In our institutional experience, analysis of endoscopic treatment results of 106 patients with hepatolithiasis has shown that the overall residual stone rate was 16%, but this rate was higher in patients with Tsunoda types III and IV (24% and 30%, respectively). We thought that this result was from the intra-hepatic stricture and the sharp angled branches of the intra-hepatic bile duct anatomy[13,14]. And it is well known that there are major limitations of endoscopic hepatolithiasis, such as parenchymal destruction of the liver, liver abscess formation, and a 5-10% incidence of cholangiocarcinoma that occurs in patients with hepatolithiasis[1,2,15,16]. Our study revealed a slightly higher rate (11%) of patients found with cholangiocarcinoma, compared to previous reports from other institutions, and the reason for this is thought to be due to the fact that patients who received surgery in our institution were those with parenchymal destruction and intra-hepatic bile duct stricture, and also because a larger number of patients were with long-standing, advanced hepatolithiasis. Another serious problem in patients with hepatolithiasis accompanied by the presence of cholangiocarcinoma is that the latter disease is not easily detected in the pre-operative evaluation, and thus may be missed during endoscopic treatment of hepatolithiasis[2,17]. This was also our finding in this study.

Endoscopic treatment of hepatolithiasis is considered to be relatively safe[18]. However, to endoscopically remove all stones completely and to resolve stricture of the intra-hepatic bile duct, the procedure is conducted on an average of 3 to 4 times at 1-2 wk intervals, restricting the patients' activities over a long period of time, even though the procedure entails less physical and psychological burden for the patient than surgical operation.

In Korea as well as in most Asian populations, pigment stones are the most common, while cholesterol stones are predominant in Western populations. This was also the case in our study in which all patients who received surgery were with pigment stones. In contrast to cholesterol stones, pigment stones demonstrate different clinicopathologic features of the intra-hepatic bile duct. Most cholesterol hepatolithiasis are usually located in the

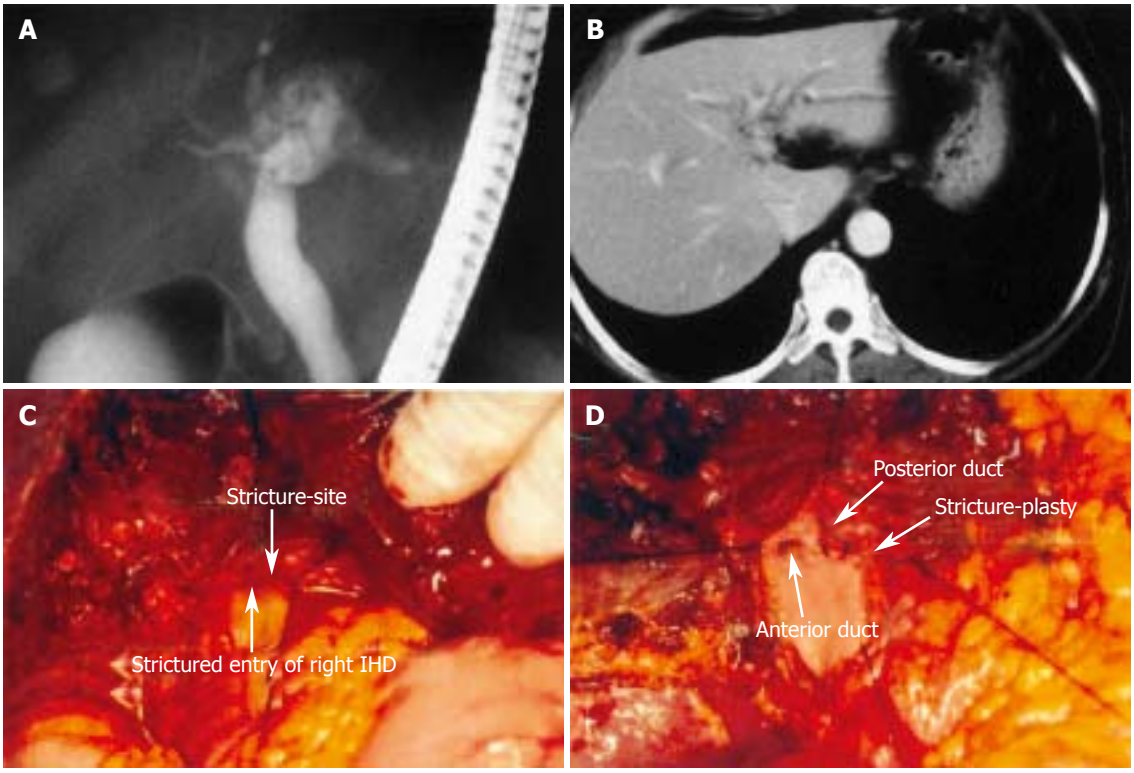

Figure 4 A and **B**: Pre-operative imaging of hepatolithiasis with hilar duct stricture; **C:** opened hilar IHD shows narrow entry of right IHD (arrow); **D:** after stricture-plasty both entries of the right anterior and posterior ducts are seen.

peripheral intra-hepatic ducts, accompanied by a lesser incidence of cholangitis, there is less wall thickening of the stone-containing duct, and the presenting symptoms are usually mild. However, pigment stones are frequently accompanied by hilar duct stricture and hilar duct stones, show severe wall thickening of the stone-bearing duct, and the symptoms are more severe due to a higher incidence of cholangitis[19]. According to our knowledge of previously reported literature, we have not been able to find any reports with regard to the rate of hilar duct stricture in hepatolithiasis patients due to pigment stone. We, however, were able to observe in our study that hilar stricture was present in 90 of 153 (59%) patients. Therefore, it is the opinion of the authors that during surgery for pigment stone hepatolithiasis, resection of the stone bearing liver parenchyma and removal of stones only is inadequate because the possibly accompanied hilar strictures will make recurrent diseases, and which must be appropriately corrected during surgery.

The VHE procedure adopted and analyzed by the authors of this study has been established as a safe method in a previous report of the pre-caudate plane of the para-caval portion of the caudate lobe[11]. Anatomically, the pre-caudate plane may be described as a plane bounded inferiorly by the hilar Glisson's pedicle, superiorly by the dorsal portion of origin of the middle hepatic vein, the left side by the ligamentum venosum, and the right side by the imaginary line of the second order branching point of the right Glisson's pedicle and the dorsal portion of origin of the right hepatic vein[11,20].

According to the experience of the authors of this study, we did not observe any postoperative incidence of severe hemorrhage of the resection plane or hypoxic damage of the caudate lobe during hepatotomy along the pre-caudate plane for left lobectomy or extended right lobectomy of the liver.

This VHE method also permitted facilitated understanding of individual hilar bile duct anatomy, the openings of second order branches from the hilar bile duct by completely exposing the ventral hilar Glisson's pedicle and then incising along the hilar bile duct. It also allowed increased accuracy of the intra-operative choledochoscopy, complete removal of intra-hepatic stones without difficulty under direct visualization, and easier stricture-plasty of the constricted hilar bile duct.

Our experience showed that the opening of hilar bile duct along its direction will allow visualization of 3 or 4 openings of the second order branches of the intra-hepatic bile duct, which emerge beginning from the right side in the following order; the anterior segmental branch, the posterior segmental branch (or common duct opening of right anterior and posterior hepatic duct), and 2 caudate branches. In many instances, the order and the number of caudate branches varied from patient to patient, and the order frequently presented as anterior segmental branch, caudate branch, posterior branch, and then the caudate branch. When hilar bile duct stricture is present, the stricture site may exist anywhere in the openings of the second order branches, thus necessitating adequate exposure of the hilar bile duct for direct visualization of the inside of the hilar bile duct for preservation of each second order branches during stricture-plasty (Figure 4).

Whenever possible, the authors did not perform a bilioenterostomy after hepatectomy but attempted to

conserve the anatomy of the bile duct physiologically. This was because according to two studies by Kusano *et al*[21] and Jan *et al*[22], incidence of cholangitis was high after bilioenterostomy.

In this study, the 5.4% rate of residual stones and 4.2% of recurrent stones after VHE surgery for hepatolithiasis were much better results than historical control datas of conventional hepatectomy that showed residual stone rate about 10% and recurrent stone rate about 12-29.6%[22-25], and this may be attributed to the high efficacy of the VHE procedure in correcting the bile duct stricture. Moreover, as the patients who underwent VHE for hepatolithiasis in this study were all high grade Tsunoda type which is difficult to remove by endoscopic methods, the effectiveness of the proposed VHE method is further stressed. We also emphasize the complication rate of VHE which was comparable with that of conventional hepatectomy.

In conclusion, we present in this study the VHE method that allows accurate assessment of the pathologic anatomy of the intra-hepatic bile duct during hepatectomy for hepatolithiasis. We also suggest from our observed data that this procedure will decrease the residual stone rate and long term recurrent stone rate after surgery without serious complications. We recommend this procedure for surgical treatment of intractable hepatolithiasis.

REFERENCES

1 **Chen MF**, Jan YY, Wang CS, Hwang TL, Jeng LB, Chen SC, Chen TJ. A reappraisal of cholangiocarcinoma in patient with hepatolithiasis. *Cancer* 1993; **71**: 2461-2465

2 **Kubo S**, Kinoshita H, Hirohashi K, Hamba H. Hepatolithiasis associated with cholangiocarcinoma. *World J Surg* 1995; **19**: 637-641

3 **Park YH**, Park SJ, Jang JY, Ahn YJ, Park YC, Yoon YB, Kim SW. Changing patterns of gallstone disease in Korea. *World J Surg* 2004; **28**: 206-210

4 **Cetta F**, Lombardo F, Cariati A. The role of the content (decreased level of apolipoprotein A1) and of the container (bile duct stricture, sectorial dilatation of the ducts determining bile stasis) in the pathogenesis of hepatolithiasis, either pigment or cholesterol. *Hepatology* 1994; **19**: 1539-1541

5 **Nakanuma Y**, Yamaguchi K, Ohta G, Terada T. Pathologic features of hepatolithiasis in Japan. *Hum Pathol* 1988; **19**: 1181-1186

6 **Hwang JH**, Yoon YB, Kim YT, Cheon JH, Jeong JB. Risk factors for recurrent cholangitis after initial hepatolithiasis treatment. *J Clin Gastroenterol* 2004; **38**: 364-367

7 **Maetani I**, Ishiguro J, Ogawa S, Sato M, Igarashi Y, Sakai Y. Percutaneous choledochoscopic treatment of intrahepatic stones, including management of associated biliary stenoses. *Endoscopy* 1999; **31**: 456-459

8 **Uchiyama K**, Onishi H, Tani M, Kinoshita H, Ueno M, Yamaue H. Indication and procedure for treatment of hepatolithiasis. *Arch Surg* 2002; **137**: 149-153

9 **Cheung MT**, Wai SH, Kwok PC. Percutaneous transhepatic choledochoscopic removal of intrahepatic stones. *Br J Surg* 2003; **90**: 1409-1415

10 **Tsunoda T**, Tsuchiya R, Harada N, Yoshino R, Noda T, Izawa K, Yamaguchi T, Yamamoto K. Long-term results of surgical treatment for intrahepatic stones. *Jpn J Surg* 1985; **15**: 455-462

11 **Kwon D**, Murakami G, Hata F, Wang HJ, Chung MS, Hirata K. Location of the ventral margin of the paracaval portion of the caudate lobe of the human liver with special reference to the configuration of hepatic portal vein branches. *Clin Anat* 2002; **15**: 387-401

12 **Sheen-Chen SM**, Cheng YF, Chou FF, Lee TY. Ductal dilatation and stenting make routine hepatectomy unnecessary for left hepatolithiasis with intrahepatic biliary stricture. *Surgery* 1995; **117**: 32-36

13 **Hwang MH**, Tsai CC, Mo LR, Yang CT, Yeh YH, Yau MP, Yueh SK. Percutaneous choledochoscopic biliary tract stone removal: experience in 645 consecutive patients. *Eur J Radiol* 1993; **17**: 184-190

14 **Cheng YF**, Lee TY, Sheen-Chen SM, Huang TL, Chen TY. Treatment of complicated hepatolithiasis with intrahepatic biliary stricture by ductal dilatation and stenting: long-term results. *World J Surg* 2000; **24**: 712-716

15 **Su CH**, Shyr YM, Lui WY, P'Eng FK. Hepatolithiasis associated with cholangiocarcinoma. *Br J Surg* 1997; **84**: 969-973

16 **Chijiiwa K**, Ohtani K, Noshiro H, Yamasaki T, Shimizu S, Yamaguchi K, Tanaka M. Cholangiocellular carcinoma depending on the kind of intrahepatic calculi in patients with hepatolithiasis. *Hepatogastroenterology* 2002; **49**: 96-99

17 **Kim YT**, Byun JS, Kim J, Jang YH, Lee WJ, Ryu JK, Kim SW, Yoon YB, Kim CY. Factors predicting concurrent cholangiocarcinomas associated with hepatolithiasis. *Hepatogastroenterology* ; **50**: 8-12

18 **Simon T**, Fink AS, Zuckerman AM. Experience with percutaneous transhepatic cholangioscopy (PTCS) in the management of biliary tract disease. *Surg Endosc* 1999; **13**: 1199-1202

19 **Kondo S**, Nimura Y, Hayakawa N, Kamiya J, Nagino M, Miyachi M, Kanai M. A clinicopathologic study of primary cholesterol hepatolithiasis. *Hepatogastroenterology* 1995; **42**: 478-486

20 **Kitagawa S**, Murakami G, Hata F, Hirata K. Configuration of the right portion of the caudate lobe with special reference to identification of its right margin. *Clin Anat* 2000; **13**: 321-340

21 **Kusano T**, Isa TT, Muto Y, Otsubo M, Yasaka T, Furukawa M. Long-term results of hepaticojejunostomy for hepatolithiasis. *Am Surg* 2001; **67**: 442-446

22 **Jan YY**, Chen MF, Wang CS, Jeng LB, Hwang TL, Chen SC. Surgical treatment of hepatolithiasis: long-term results. *Surgery* 1996; **120**: 509-514

23 **Sato M**, Watanabe Y, Horiuchi S, Nakata Y, Sato N, Kashu Y, Kimura S. Long-term results of hepatic resection for hepatolithiasis. *HPB Surg* 1995; **9**: 37-41

24 **Chijiiwa K**, Kameoka N, Komura M, Yamasaki T, Noshiro H, Nakano K. Hepatic resection for hepatolithiasis and long-term results. *J Am Coll Surg* 1995; **180**: 43-48

25 **Fan ST**, Lai EC, Wong J. Hepatic resection for hepatolithiasis. *Arch Surg* 1993; **128**: 1070-1074

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RAPID COMMUNICATION

Differential c-erbB-1 and c-erbB-2 mRNA expression in cancer of the pancreas compared with cancer of the papilla of Vater

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Abstract

AIM: We examined quantitative mRNA expression of growth factor receptors (c-erbB-1, c-erbB-2) and the anti-apoptosis gene survivin known to be regulated in pancreatic adenocarcinomas and compared the expression pattern with that in carcinomas of the papilla of Vater.

METHODS: Quantitative real-time reverse transcriptase-PCR (QRT-PCR, Taqman™) was performed to analyze mRNA expression levels of c-erbB-1, c-erbB-2 and survivin in normal and corresponding tumor samples of 31 pancreatic adenocarcinomas and 8 cancers of the papilla of Vater.

RESULTS: The overall median mRNA expression of survivin was significantly increased in both adenocarcinoma of the pancreas ($P<0.01$) and papilla of Vater ($P<0.008$) compared with uninvolved normal control tissue. In pancreatic cancer, expression of c-erbB-1 was significantly decreased compared with the normal pancreatic tissue ($P<0.03$), whereas in the cancer of the papilla of Vater expression of c-erbB-2 was significantly downregulated ($P<0.05$) compared with the paired normal samples. Gene expression was not associated with tumor stage, differentiation or prognosis.

CONCLUSION: The common anti-apoptosis gene survivin is overexpressed both in the cancer of the papilla of Vater and pancreas. In contrast, the growth factor receptor genes c-erbB-1 and c-erbB-2 are differentially regulated in both tumor entities adding further evidence that pancreatic cancer is biologically different from the cancer of papilla of Vater.

INTRODUCTION

Adenocarcinoma of the pancreas has the worst prognosis of all cancers, with a 5-year survival rate less than 3%, accounting for the fifth highest number of cancer related deaths in North America and Europe with a rising incidence[1-3]. The only curative treatment for pancreatic cancer (PC) is complete resection which is possible in only 10-20% of patients[4] and associated with relatively constant 5-year survival rates of approximately 20% for the last 30 years[5,6,7]. The dismal prognosis and the lack of effective therapeutic regimens for this disease are attributed to several causes: (a) PC shows an aggressive biological phenotype, which is characterized by the early invasion of surrounding structures and rapid metastatic spread[8,9]; (b) radiation therapy and chemotherapy are reported to be effective only in selected patients after tumor resection[9-11]; (c) although various genetic alterations were identified, which have improved our understanding of the carcinogenesis of this disease, the responsible molecular mechanisms are largely unknown. New treatment regimens based on molecular classifications of the individual tumor may improve the outcome for patients with PC.

In contrast, cancer of the papilla of Vater (CPV) has a better prognosis with a 5-year survival rate of over 40% after curative resection[12,13]. CPV is a rare disease representing 6-12% of all periampullary malignancies[14] with an estimated incidence of 2.9 per million[15]. Because of its anatomical location, CPV becomes symptomatic at an earlier stage than PC. Therefore, the majority of the patients with CPV are candidates for surgical therapy. Evidence is increasing that differences in the tumor

biology of these two entities contribute to the different prognosis[16].

Survivin is a member of the inhibitor of apoptosis gene family, which is unique for its expression in a wide range of embryonic and fetal tissues, whereas almost no transcripts are detected in terminally differentiated normal adult tissues. In human pancreatic islets survivin expression is also developmentally regulated[17] . Survivin expression in human fetal islets was identified immunohistochemically in alpha and beta islet cells whereas adult pancreases showed a staining only in the alpha cells. However, it is re-expressed in several human cancers[18] and has an additional function in the regulation of cellular proliferation[19] and angiogenesis[20] of cancers. Survivin expression has been reported to be associated with poor survival in patients with colorectal cancer[21,22] and several other human cancers[23]. In a recent immunohistochemical study on 52 PC patients, increased survivin expression was strongly correlated to a higher proliferative index, nevertheless no correlation between survivin expression and survival of the patients was shown[24].

The epidermal growth factor receptor (EGF-R) family contains four structurally homologous transmembrane proteins with intracellular tyrosine kinase activity. The best-described growth factor receptors of this family are EGF-R also known as c-erbB-1[25] and the c-erbBs-2, 3, and 4[26]. They share a significant sequence homology and are frequently overexpressed in PC. Activation of the receptors leads to increased DNA synthesis, and changes in cell motility and cell metabolism[27]. EGF-R is activated by a family of peptide ligands that includes EGF, TGF-α, heparin binding EGF-like growth factor, betacellulin, and amphiregulin[28]. The EGF-R is encoded by the c-erbB-1 proto-oncogene and is a transmembrane growth factor with tyrosin kinase activity. In normal pancreas, c-erbB-1 is expressed only in the islets of Langerhans. Nevertheless the c-erbB-1 gene is overexpressed in human pancreatic cell lines and in 95% of ductal adenocarcinomas, due to an increase in gene transcription[29]. Overexpression of c-erbB-1 detected by immunohistochemistry is correlated with reduced survival rates in several studies[30,31] .

The c-erbB-2 (also called HER2/neu) gene encodes a 185-ku transmembrane glycoprotein with tyrosine kinase activity, and acts as a receptor for a class of ligands that includes the heregulins, gp30, and NEU-differentiation factor. C-erbB-2 is overexpressed in the bladder, breast, esophageal, and gastric cancer, where it appears to have a role in lymphatic tumor spread[32,33]. In ductal adenocarcinomas of the pancreas and in ampullary tumors, c-erbB-2 is overexpressed in 20%, which is usually caused by gene amplification[34]. Reports about the prognostic significance of c-erbB-2 are not uniform throughout the literature. One study showed a survival advantage for patients without the overexpression of c-erbB-2 in their tumors[35] , while another study showed a correlation of c-erbB-2 serum levels and survival of the patients [36]. In the majority of studies no prognostic significance for the expression of c-erbB-2 in PC was revealed[37,38].

In the present study we have determined gene expression levels of survivin, c-erbB-1 and c-erbB-2 in PC and CPV applying quantitative real-time RT-PCR (TaqMan ™) in order to look for gene profiles that might account for the different biological behavior.

MATERIALS AND METHODS

Patients and specimens

Between July 1997 and December 2003 fresh frozen tumor and corresponding normal tissue from 63 patients, who underwent curative resection of suspected or proven pancreatic or ampullary tumors were collected. Informed consent was obtained from all the patients. Data and tissue collection were in accordance with the regulation of the local ethic committee. Tissues were frozen immediately in liquid nitrogen and stored at -80 °C until further analysis.

The definitive histology of the tissue used for RNA isolation was confirmed in serial sections by a staff pathologist. Only tumor specimens with at least 75% malignant cells were used for RNA isolation.

Finally, 31 specimens from ductal adenocarcinomas of the pancreas and 8 from adenocarcinomas of the papilla of Vater with paired normal control tissues were available for gene expression analysis. This study population consisted of 24 (62%) men and 15 (38%) women with a median age of 59.4 years (range, 33-81 years). Tumor staging was performed according to the International Union Against Cancer (UICC) tumor-node-metastasis classification. In patients with PC, 1 (3.2%) patient had a stage I tumor, 3 (9.6%) patients had a stage II tumor, 24 (77.4%) patients had a stage III, and 3 (9.6%) had a stage IVa tumor. In patients with CVP, 5 patients presented with a stage II tumor, 2 patients had stage III, and 1 patient a stage IV tumor.

Twenty four patients underwent a Kausch-Whipple procedure, and in 10 patients the pylorus was preserved. In four patients a left-sided pancreatic resection and in one patient a pancreatectomy was performed. In patients with cancer of the papilla (n=8) of Vater, five received a pylorus preserving partial pancreaticoduodenectomy, whereas three patients underwent a Kausch-Whipple procedure. The median follow-up for surviving patients was 9.5 mo and no patient was lost to follow-up.

RNA isolation

Total cellular RNA was isolated using RNeasy Mini Kit (Qiagen, Hilden) adding proteinase K (0.2 mg/mL) and quantified at $A_{260/280\ nm}$ (SmartSpec; Bio-Rad, Hercules, CA, USA).

Quantitative RT-PCR

Total cellular RNA (0.5 μg) was reverse-transcribed as described previously[39]. An amount of 25 ng of cDNA was taken for real-time PCR using the TaqMan ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Darmstadt, Germany). To normalize the amount of total RNA present in each reaction, the housekeeping gene β-actin was amplified.

Primers and probes were designed to encompass intron between exon sequences using the Primer Express Software (Applied Biosystems, Darmstadt, Germany). The β-actin probe was labeled with 5′-VIC and 3′-Minor Groove Binder/Non-Fluorescent Quencher (Applied

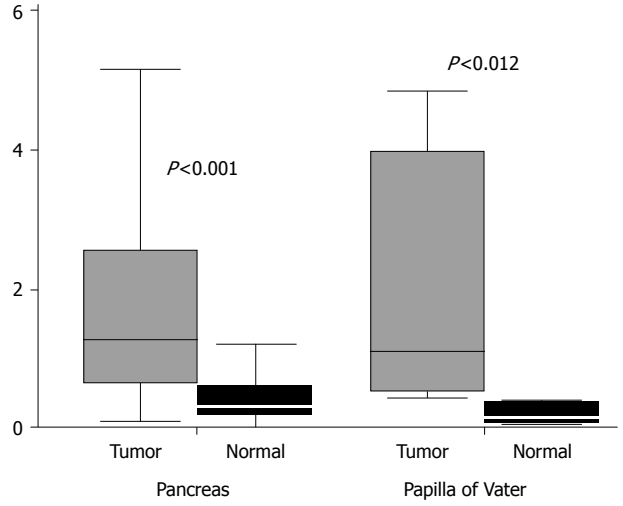

Figure 1 Expression of survivin in PC (n=31) and CPV (n=8).

Biosystems, Darmstadt, Germany). The survivin, c-erb-B-1 and c-erbB-2 probes were labeled at the 5′ end with FAM and at the 3′ end with the quencher TAMRA (Eurogentec, Seraing, Belgium). The sequences for the primers and probes were as follows: survivin described by Warnecke-Eberz *et al* 2005. C-erbB-2 sense 5′ CCA GGA CCT GCT GAA CTG GT 3′, anti-sense 5′ TGT ACG AGC CGC ACA TCC 3′, probe 5′ CAG TTG CCA AGG GGA TGA GCT ACC TG 3′. C-erbB-1 sense 5′ CGC AAG TGT AAG AGT GCG AA 3′, anti-sense 5′ CGT AGC ATT TAT GGA GAG TGA GTC T 3′, probe 5′ CCT TGC CGC AAA GTG TGT AAC GGA AT 3′. The reliability of PCR amplification and detection was verified on serial dilutions of standard cDNAs prior to analyses of patient samples. To ensure that no genomic DNA was amplified the assays were checked with RNA samples minus reverse transcription control as well as with genomic DNA as template.

The PCR reaction was performed as described previously[39]. All analyses were done in triplicates. Gene expression levels were calculated using standard curves generated by serial dilutions of placenta cDNA (Clontech Laboratories, Palo Alto, CA, USA).

Statistical analysis

The gene expression analyses yielded values, which were expressed as ratios between two absolute measurements: the gene of interest and the internal reference gene β-actin. Gene expression levels were described using the median as point estimator and the range of values. Cut-off values for discrimination of dichotomized mRNA expression levels and clinicopathologic parameters were derived from receiver operating curve data (area under the curve and the 95% confidence interval) according to Metz *et al*[40]. Associations between gene expression levels and clinicopathological parameters were evaluated using the χ^2 test for dichotomized variables, Wilcoxon's rank test for paired variables and the Mann-Whitney test for independent variables applying Fisher's exact testing for significance.

Partitioning of gene expression levels to construct prognostic groups was performed according to LeBlanc *et al*[41]. Briefly, the best cut-off value for a supposed prognostic variable is determined by simulating the log-rank test for all observed covariate values within the entire data set. The minimal log-rank P-value determines the best cut-off value for dichotomization of the covariate. Kaplan-Meier[42] plots were used to describe the survival distribution and the log-rank test was used to evaluate survival differences[43].

The level of significance was set at $P<0.05$ in all statistical testing. Unless otherwise specified, P-values were given for two-sided testing.

Table 1 Results of non-parametric paired samples Wilcoxon's test. mRNA expression of c-erbB-1 and c-erbB-2 are compared between tumor (T) and normal (N) samples

Tumor		c-erbB-1	*P*	c-erbB-2	*P*
Pancreatic cancer	T<N	21	0.03	14	0.99
	T>N	9		17	
Cancer of the papilla of Vater	T<N	5	0.38	6	<0.05
	T>N	3		2	

RESULTS

Survivin mRNA expression

Survivin mRNA expression was detected in 30 of 31 (97%) PC in all tumor specimens of the papilla of Vater and in all paired non-malignant control specimens. In 26/31 (83%) PC expression of survivin mRNA was higher in tumor compared to normal control tissue and this difference was statistically significant (Wilcoxon's test: $P<0.001$). In CVP, all tumor specimens (n=8) had a higher expression than the normal tissue (Wilcoxon's test: $P<0.01$). Expression of survivin mRNA was not significantly different between PC and CPV (Mann-Whitney test: $P=0.195$). Representative box-plots are shown in Figure 1.

C-erbB-1 mRNA expression

C-erbB-1 mRNA expression could be detected in 30 of 31 (97%) pancreatic tumors, all tumors of the papilla and all normal tissues. Expression of c-erbB-1 was lower in the malignant than in the normal pancreatic tissue in 21 out of 31 (68%) individual cases. This difference was statistically significant (Wilcoxon's test: $P=0.028$), whereas in CPV no statistically significant difference ($P=0.38$) in c-erbB-1 expression between tumor and corresponding normal tissue was revealed (Table 1).

C-erbB-2 mRNA expression

The expression of c-erbB-2 was detectable in all tumor and non-malignant specimens. In PC paired non-parametric testing revealed no statistically significant difference in c-erbB-2 expression between tumor and normal tissue. In contrast, c-erbB-2 expression was significantly (Wilcoxon's test: $P<0.05$) downregulated in 6/8 cases of CPV (Table 1).

No significant association between gene expression levels and clinical parameters such as sex (χ^2 test), tumor stage, lymph node stage, and grade of differentiation (Mann-Whitney test) was observed.

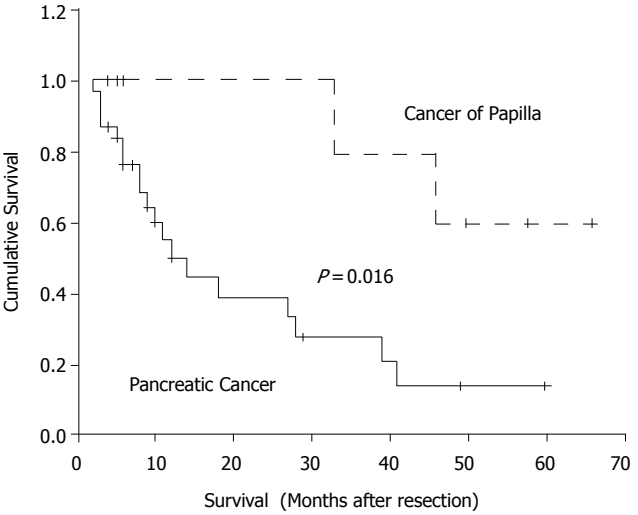

Figure 2 Kaplan–Meier analysis of survival of patients with pancreatic cancer ($n = 31$) and CPV ($n = 8$) after resection.

mRNA expression and survival of patients with pancreatic cancer and cancer of the ampulla of Vater

At a median follow-up of 56 months (range 7-89 mo) for surviving patients, median survival was 14 mo (range 2-60 mo) for patients with pancreatic carcinoma and 39.5 months (range 4-66 mo) for patients with carcinoma of the papilla of Vater (log-rank: $P<0.016$). Kaplan-Meier survival curves are shown in Figure 2.

Partitioning of gene expression levels to construct prognostic groups according to LeBlanc *et al*[41] did not reveal any correlation between gene expression (c-erbB-1, c-erbB-2, and survivin) and survival of patients with pancreatic or papillary cancer.

DISCUSSION

The surgical therapy of carcinoma of the papilla of Vater and adenocarcinoma of the pancreas consists of a pylorus preserving or a classical pancreaticoduodenectomy (Whipple procedure). Despite this identical surgical approach, the long-term prognosis is different. Five-year survival rates from 0% to 25% for PC and from 15% to 56% for CPV have been reported[1,12]. It was shown that different tumor biology, represented by higher expression of members of the EGF-R family (c-erbB-1, c-erbB-2, and c-erbB-3) contributes to the different behavior[16,44]. C-erbB-1 and c-erbB-2 were also differentially expressed in our study. In contrast to Friess *et al*[16], our results showed a significant downregulation of c-erbB-1 expression in PC tissue and of c-erbB-2 expression in the malignant tissue of the papilla of Vater compared with the adjacent normal tissues. These results were based on a smaller number of patients, but were obtained by quantitative real time RT-PCR. Whereas the above mentioned study[16] applied immunohistochemical staining and Northern blot analysis, quantitative real time RT-PCR provides more accurate and reproducible quantitation of gene expression and has a large range of results[45,46].

The downregulation of c-erbB-1 in malignant pancreatic tissues compared with the adjacent normal tissue is in contrast to the existing literature that shows an overexpression of c-erbB-1 in pancreatic ductal adenocarcinoma and a worse prognosis for patients with high expression of c-erbB-1 in their tumors[29,30,47,48]. As mentioned above, these studies, which were mainly published about 10 years ago, were based on immunohistochemical techniques showing c-erbB-1 expression in only 30-70% of the investigated PC cases. Importantly, the results were not compared with the matching normal pancreatic tissue from the same patients. In our investigation using real time RT-PCR, c-erbB-1 expression was detected in 97% of PCs and in all matching normal pancreatic samples.

The reported expression of c-erbB-2 in PC has varied widely (7-82%) in different studies due to the differences in methodology and/or patient selection[35-38]. Our study, which is the first in using quantitative real-time RT-PCR to assess the expression of c-erbB-2 in pancreatic and CPV, did not show a different expression of c-erbB-2 between tumor and normal pancreatic tissue. This is in agreement with two recent studies[49,50] using immunohistochemical methods, which found no correlation between c-erbB-2 expression and stage or survival of patients with PC. However, a series of other publications[45-48,51] reported overexpression of c-erbB-2 in a subset of patients with PC and its prognostic relevance. For other malignancies such as gastric cancer[52], breast cancer[53], and ovarian cancer[54] an increased expression of the c-erbB-2 oncogene has been reported, applying PCR based methods. According to our results and the recent literature one must consider, that the role of c-erbB-2 in the tumorigenesis and its value as a prognostic marker in PC has not been finally elucidated.

In concordance with other authors[24,55-57], we found an increased expression of survivin in the samples of PC and CPV compared with the adjacent non-malignant pancreas. One study[24] also found an overexpression of survivin in a series of 12 ampullary carcinomas; expression was not different from PC, which is comparable with our result. Kami *et al*[58] reported a significant correlation between poor survival and increased expression of survivin in a study with 47 patients with PC using immunohistochemistry. Whereas Sarela *et al*[24], as well as our study revealed no association between survival and expression of survivin. The comparable results of survivin expression in our patients and other studies in the literature strengthen our data regarding the expression of c-erbB-1 and c-erbB-2 in PC and CPV. For a variety of other malignancies it was shown that survivin expression is a reliable marker for an unfavorable disease with poor overall survival[59,60]. The high number of locally advanced tumors, on which our and other studies are based, could explain the difference to PC. The significantly increased expression of survivin in PC, obtained by different methodologies, opens possibilities for new diagnostic and therapeutic strategies, such as new targets for gene therapy, differentiation between malignant and benign pancreatic lesions before surgical resection and to monitor response to neoadjuvant treatment regimens.

REFERENCES

1 **Jemal A**, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ.

Cancer statistics, 2003. *CA Cancer J Clin* 2003; **53**: 5-26
2 **Fernandez E**, La Vecchia C, Porta M, Negri E, Lucchini F, Levi F. Trends in pancreatic cancer mortality in Europe, 1955-1989. *Int J Cancer* 1994; **57**: 786-792
3 **Bramhall SDJ**, Neoptolemos JP. Epidemiology of pancreatic cancer. Vol. 2. Boston, MA: Blackwell Scientific, 1998: 889-906
4 **Yeo CJ**, Cameron JL, Sohn TA, Lillemoe KD, Pitt HA, Talamini MA, Hruban RH, Ord SE, Sauter PK, Coleman J, Zahurak ML, Grochow LB, Abrams RA. Six hundred fifty consecutive pancr eaticoduodenectomies in the 1990s: pathology, complications, and outcomes. *Ann Surg* 1997; **226**: 248-57; discussion 257-60
5 **Hawes RH**, Xiong Q, Waxman I, Chang KJ, Evans DB, Abbruzzese JL. A multispecialty approach to the diagnosis and management of pancreatic cancer. *Am J Gastroenterol* 2000; **95**: 17-31
6 **Bramhall SR**, Allum WH, Jones AG, Allwood A, Cummins C, Neoptolemos JP. Treatment and survival in 13,560 patients with pancreatic cancer, and incidence of the disease, in the West Midlands: an epidemiological study. *Br J Surg* 1995; **82**: 111-115
7 **Sener SF**, Fremgen A, Menck HR, Winchester DP. Pancreatic cancer: a report of treatment and survival trends for 100,313 patients diagnosed from 1985-1995, using the National Cancer Database. *J Am Coll Surg* 1999; **189**: 1-7
8 **Magee CJ**, Greenhalf W, Howes N, Ghaneh P, Neoptolemos JP. Molecular pathogenesis of pancreatic ductal adenocarcinoma and clinical implications. *Surg Oncol* 2001; **10**: 1-23
9 **Greenlee RT**, Hill-Harmon MB, Murray T, Thun M. Cancer statistics, 2001. *CA Cancer J Clin* 2001; **51**: 15-36
10 **Neoptolemos JP**, Stocken DD, Dunn JA, Almond J, Beger HG, Pederzoli P, Bassi C, Dervenis C, Fernandez-Cruz L, Lacaine F, Buckels J, Deakin M, Adab FA, Sutton R, Imrie C, Ihse I, Tihanyi T, Olah A, Pedrazzoli S, Spooner D, Kerr DJ, Friess H, Büchler MW. Influence of resection margins on survival for patients with pancreatic cancer treated by adjuvant chemoradiation and/or chemotherapy in the ESPAC-1 randomized controlled trial. *Ann Surg* 2001; **234**: 758-768
11 **Sohn TA**, Yeo CJ, Cameron JL. Resected adenocarcinoma of the pancreas -616 patients: results, outcomes, and prognostic indicators. *J Gastrointestinal Surg* 2000; **4**: 567-579
12 **Beger HG**, Treitschke F, Poch B. Adenocarcinoma of the ampulla of Vater-operative treatments and results. In: Beger HG, Warshaw Al, Büchler MW. The Pancreas. Oxford: Blackwell Science, 1998: 1328-1331
13 **Willett CG**, Warshaw AL, Convery K, Compton CC. Patterns of failure after pancreaticoduodenectomy for ampullary carcinoma. *Surg Gynecol Obstet* 1993; **176**: 33-38
14 **Neoptolemos JP**, Talbot IC, Carr-Locke DL, Shaw DE, Cockleburgh R, Hall AW, Fossard DP. Treatment and outcome in 52 consecutive cases of ampullary carcinoma. *Br J Surg* 1987; **74**: 957-961
15 **Anderson JB**, Cooper MJ, Williamson RC. Adenocarcinoma of the extrahepatic biliary tree. *Ann R Coll Surg Engl* 1985; **67**: 139-143
16 **Friess H**, Wang L, Zhu Z, Gerber R, Schröder M, Fukuda A, Zimmermann A, Korc M, Büchler MW. Growth factor receptors are differentially expressed in cancers of the papilla of vater and pancreas. *Ann Surg* 1999; **230**: 767-74; discussion 774-5
17 **Liggins C**, Orlicky DJ, Bloomquist LA, Gianani R. Developmentally regulated expression of Survivin in human pancreatic islets. *Pediatr Dev Pathol* 2003; **6**: 392-397
18 **Ambrosini G**, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med* 1997; **3**: 917-921
19 **Li F**, Ambrosini G, Chu EY. Control of apoptosis and mitotic spindle check point by survivin. *Nature* 1999; **396**: 580-594
20 **O'Connor DS**, Schechner JS, Adida C, Mesri M, Rothermel AL, Li F, Nath AK, Pober JS, Altieri DC. Control of apoptosis during angiogenesis by survivin expression in endothelial cells. *Am J Pathol* 2000; **156**: 393-398
21 **Sarela AI**, Macadam RC, Farmery SM, Markham AF, Guillou PJ. Expression of the antiapoptosis gene, survivin, predicts death from recurrent colorectal carcinoma. *Gut* 2000; **46**: 645-650
22 **Sarela AI**, Scott N, Ramsdale J, Markham AF, Guillou PJ. Immunohistochemical detection of the anti-apoptosis protein, survivin, predicts survival after curative resection of stage II colorectal carcinomas. *Ann Surg Oncol* 2001; **8**: 305-310
23 **Altieri DC**, Marchisio PC. Survivin apoptosis: an interloper between cell death and cell proliferation in cancer. *Lab Invest* 1999; **79**: 1327-1333
24 **Sarela AI**, Verbeke CS, Ramsdale J, Davies CL, Markham AF, Guillou PJ. Expression of survivin, a novel inhibitor of apoptosis and cell cycle regulatory protein, in pancreatic adenocarcinoma. *Br J Cancer* 2002; **86**: 886-892
25 **Ullrich A**, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. *Cell* 1990; **61**: 203-212
26 **Carraway KL**, Cantley LC. A neu acquaintance for erbB3 and erbB4: a role for receptor heterodimerization in growth signaling. *Cell* 1994; **78**: 5-8
27 **Petch LA**, Harris J, Raymond VW, Blasband A, Lee DC, Earp HS. A truncated, secreted form of the epidermal growth factor receptor is encoded by an alternatively spliced transcript in normal rat tissue. *Mol Cell Biol* 1990; **10**: 2973-2982
28 **Prigent SA**, Lemoine NR. The type 1 (EGFR-related) family of growth factor receptors and their ligands. *Prog Growth Factor Res* 1992; **4**: 1-24
29 **Barton CM**, Hall PA, Hughes CM, Gullick WJ, Lemoine NR. Transforming growth factor alpha and epidermal growth factor in human pancreatic cancer. *J Pathol* 1991; **163**: 111-116
30 **Yamanaka Y**, Friess H, Kobrin MS, Buchler M, Beger HG, Korc M. Coexpression of epidermal growth factor receptor and ligands in human pancreatic cancer is associated with enhanced tumor aggressiveness. *Anticancer Res* 1993; **13**: 565-569
31 **Uegaki K**, Nio Y, Inoue Y, Minari Y, Sato Y, Song MM, Dong M, Tamura K. Clinicopathological significance of epidermal growth factor and its receptor in human pancreatic cancer. *Anticancer Res* 1997; **17**: 3841-3847
32 **Akiyama T**, Sudo C, Ogawara H, Toyoshima K, Yamamoto T. The product of the human c-erbB-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. *Science* 1986; **232**: 1644-1646
33 **Mellon JK**, Lunec J, Wright C, Horne CH, Kelly P, Neal DE. C-erbB-2 in bladder cancer: molecular biology, correlation with epidermal growth factor receptors and prognostic value. *J Urol* 1996; **155**: 321-326
34 **Hardwick RH**, Barham CP, Ozua P, Newcomb PV, Savage P, Powell R, Rahamin J, Alderson D. Immunohistochemical detection of p53 and c-erbB-2 in oesophageal carcinoma; no correlation with prognosis. *Eur J Surg Oncol* 1997; **23**: 30-35
35 **Hall PA**, Hughes CM, Staddon SL, Richman PI, Gullick WJ, Lemoine NR. The c-erb B-2 proto-oncogene in human pancreatic cancer. *J Pathol* 1990; **161**: 195-200
36 **Lei S**, Appert HE, Nakata B, Domenico DR, Kim K, Howard JM. Overexpression of HER2/neu oncogene in pancreatic cancer correlates with shortened survival. *Int J Pancreatol* 1995; **17**: 15-21
37 **Okada N**, Ohshio G, Yamaki K, Imamura T, Imamura M. Elevated serum c-erbB-2 protein levels in patients with pancreatic cancer: correlation to metastasis and shorter survival. *Oncology* 1995; **52**: 392-396
38 **Kawesha A**, Ghaneh P, Andrén-Sandberg A, Ograed D, Skar R, Dawiskiba S, Evans JD, Campbell F, Lemoine N, Neoptolemos JP. K-ras oncogene subtype mutations are associated with survival but not expression of p53, p16(INK4A), p21(WAF-1), cyclin D1, erbB-2 and erbB-3 in resected pancreatic ductal adenocarcinoma. *Int J Cancer* 2000; **89**: 469-474
39 **Warnecke-Eberz U**, Hokita S, Xi H, Higashi H, Baldus SE, Metzger R, Brabender J, Bollschweiler E, Mueller RP, Dienes HP, Hoelscher AH, Schneider PM. Overexpression of survivin mRNA is associated with a favorable prognosis following neoadjuvant radiochemotherapy in esophageal cancer. *Oncol Rep* 2005; **13**: 1241-1246
40 **Metz CE**, Goodenough DJ, Rossmann K. Evaluation of receiver operating characteristic curve data in terms of information theory, with applications in radiography. *Radiology* 1973; **109**: 297-303
41 **LeBlanc M**, Jacobson J, Crowley J. Partitioning and peeling for

constructing prognostic groups. *Stat Methods Med Res* 2002; **11**: 247-274
42 **Kaplan EL**, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc* 1958; **53**: 187-220
43 **Mantel N**. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep* 1966; **50**: 163-170
44 **Friess H**, Guo XZ, Tempia-Caliera AA, Fukuda A, Martignoni ME, Zimmermann A, Korc M, Büchler MW. Differential expression of metastasis-associated genes in papilla of vater and pancreatic cancer correlates with disease stage. *J Clin Oncol* 2001; **19**: 2422-2432
45 **Gibson UE**, Heid CA, Williams PM. A novel method for real time quantitative RT-PCR. *Genome Res* 1996; **6**: 995-1001
46 **Heid CA**, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. *Genome Res* 1996; **6**: 986-994
47 **Ghaneh P**, Kawesha A, Evans JD, Neoptolemos JP. Molecular prognostic markers in pancreatic cancer. *J Hepatobiliary Pancreat Surg* 2002; **9**: 1-11
48 **Ueda S**, Ogata S, Tsuda H, Kawarabayashi N, Kimura M, Sugiura Y, Tamai S, Matsubara O, Hatsuse K, Mochizuki H. The correlation between cytoplasmic overexpression of epidermal growth factor receptor and tumor aggressiveness: poor prognosis in patients with pancreatic ductal adenocarcinoma. *Pancreas* 2004; **29**: e1-e8
49 **Koka V**, Potti A, Koch M, Fraiman G, Mehdi S, Levitt R. Role of immunohistochemical identification of Her-2/neu and detection of variability in overexpression in pancreatic carcinoma. *Anticancer Res* 2002; **22**: 1593-1597
50 **Tamiolakis D**, Venizelos I, Simopoulos C, Kotini A, Jivannakis T, Papadopoulos N. Correlation of immunohistochemically detected HER-2/neu (c-erbB-2) with histological stage and perineural invasion in pancreatic cancer. *Hepatogastroenterology* 2004; **51**: 334-337
51 **Safran H**, Steinhoff M, Mangray S, Rathore R, King TC, Chai L, Berzein K, Moore T, Iannitti D, Reiss P, Pasquariello T, Akerman P, Quirk D, Mass R, Goldstein L, Tantravahi U. Overexpression of the HER-2/neu oncogene in pancreatic adenocarcinoma. *Am J Clin Oncol* 2001; **24**: 496-499
52 **Tsugawa K**, Fushida S, Yonemura Y. Amplification of the c-erbB-2 gene in gastric carcinoma: correlation with survival. *Oncology* 1993; **50**: 418-425
53 **Hanna WM**, Kahn HJ, Pienkowska M, Blondal J, Seth A, Marks A. Defining a test for HER-2/neu evaluation in breast cancer in the diagnostic setting. *Mod Pathol* 2001; **14**: 677-685
54 **Seki A**, Yoshinouchi M, Seki N, Kodama J, Miyagi Y, Kudo T. Detection of c-erbB-2 and FGF-3 (INT-2) gene amplification in epithelial ovarian cancer. *Int J Oncol* 2000; **17**: 103-106
55 **Yamamoto T**, Tanigawa N. The role of survivin as a new target of diagnosis and treatment in human cancer. *Med Electron Microsc* 2001; **34**: 207-212
56 **Asanuma K**, Tsuji N, Endoh T, Yagihashi A, Watanabe N. Survivin enhances Fas ligand expression via up-regulation of specificity protein 1-mediated gene transcription in colon cancer cells. *J Immunol* 2004; **172**: 3922-3929
57 **Satoh K**, Kaneko K, Hirota M, Masamune A, Satoh A, Shimosegawa T. Expression of survivin is correlated with cancer cell apoptosis and is involved in the development of human pancreatic duct cell tumors. *Cancer* 2001; **92**: 271-278
58 **Kami K**, Doi R, Koizumi M, Toyoda E, Mori T, Ito D, Fujimoto K, Wada M, Miyatake S, Imamura M. Survivin expression is a prognostic marker in pancreatic cancer patients. *Surgery* 2004; **136**: 443-448
59 **Qiao JG**, Zhang YQ, Yin YC, Tan Z. Expression of Survivin in pancreatic cancer and its correlation to expression of Bcl-2. *World J Gastroenterol* 2004; **10**: 2759-2761
60 **Altieri DC**. Survivin, versatile modulation of cell division and apoptosis in cancer. *Oncogene* 2003; **22**: 8581-8589

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RAPID COMMUNICATION

Polymorphisms in interleukin-10 gene according to mutations of *NOD2/CARD15* gene and relation to phenotype in Spanish patients with Crohn's disease

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Abstract

AIM: To examine the contribution of interleukin-10 (IL-10) gene polymorphisms to Crohn's disease (CD) phenotype, and the possible genetic epistasis between IL-10 gene polymorphisms and *CARD15/NOD2* gene mutations.

METHODS: A cohort of 205 Spanish unrelated patients with Crohn's disease recruited from a single center was studied. All patients were rigorously phenotyped and followed-up for at least 3 years (mean time, 12.5 years). The clinical phenotype was established prior to genotyping.

RESULTS: The correlation of genotype-Vienna classification groups showed that the ileocolonic location was significantly associated with the -1082G allele in the *NOD2/CARD15* mutation-positive patients (*RR* = 1.52, 95%*CI*, 1.21 to 1.91, *P* = 0.008). The multivariate analysis demonstrated that the IL-10 G14 microsatellite allele in the *NOD2/CARD15* mutation positive patients was associated with two risk factors, history of appendectomy (*RR* = 2.15, 95%CI = 1.1-4.30, *P* = 0.001) and smoking habit at diagnosis (*RR* = 1.29, 95%*CI* = 1.04-4.3, *P* = 0.04).

CONCLUSION: In Spanish population from Madrid, in CD patients carrying at least one *NOD2/CARD15* mutation, the -1082G allele is associated with ileocolonic disease and the IL-10G14 microsatellite allele is associated with previous history of appendectomy and smoking habit at diagnosis. These data provide further molecular evidence for a genetic basis of the clinical heterogeneity of CD.

Key words: Crohn´s disease; *NOD2/CARD15* gene; Interleukin-10 gene

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INTRODUCTION

Crohn's disease (CD) is a chronic inflammatory disorder of the gastrointestinal tract. The inflammation may involve any segment of the digestive tract, from the mouth to the anus, and may affect mucosa and deeper layers of the digestive wall, with or without granulomas. The etiopathogenesis of the disease remains poorly understood. Experimental and observational data suggest that intestinal inflammation arise from abnormal immune reactivity to bacterial flora in the intestine of individuals who are genetically susceptible[1].

Epidemiologic and linkage studies suggest that genetic factors play a significant role in determining CD susceptibility. CD has no simple Mendelian pattern of inheritance. As other immune diseases, CD is thought to be a heterogeneous, complex polygenic disease, where both genetic and environmental factors play an important role in the disease and, in which multiple interactions between susceptibility and resistance alleles are involved in disease pathogenesis[2,3].

Human genetic studies, notably the landmark identification in 2001 of *NOD2/CARD15* within the linkage region IBD1, have confirmed a genetic influence on CD[4-6], and it is now clear that a genotype-phenotype relationship exists. In our population of Spanish CD

patients from Madrid, mutations in the *NOD2/CARD15* gene were a marker of susceptibility to disease and were associated with ileal disease[7].

In CD, the mucosal inflammation is associated with an exaggerated and prolonged immune response because of a dysregulated production and interaction of pro-inflammatory and anti-inflammatory cytokines and their receptors[8]. A variety of genes encoding various proteins involved in the immune regulation have been postulated as possible candidates for disease susceptibility, including, among others, cytokines as interleukin-10 (IL-10). IL-10 is a regulatory cytokine that has several functions, but one important role is to act as an inhibitor of development of Th1 cells, activated macrophages and their products interleukin-12 (IL-12), tumor necrosis factor (TNF) and interferon-gamma (IFN-γ). Even though it is usually considered an inhibitory cytokine, it also has stimulatory effects (*e.g.* stimulating B cell proliferation)[9]. Recently, we have also shown that IL-10 polymorphisms contribute to susceptibility to CD in our Spanish population. IL-10G14 microsatellite allele as well as -1082G allele (guanine at position -1082) were significantly increased in Crohn´s disease patients. The combined presence of both alleles in one individual notably increased the risk to develop CD[10].

Although the pathogenetic mechanisms mediated by *NOD2/CAR15* remain elusive, it has recently been shown that one of the mutations in the *NOD2/CARD15* gene results in defective release of IL-10 from blood mononuclear cells after stimulation with Toll-like receptor (TLR) 2 ligands and this could contribute to the overwhelming inflammation seen in CD[11].

As IL-10 polymorphisms appear to confer a risk to develop CD in the Spanish population, the present study examined genotype-phenotype correlations in the disease process. Moreover, after stratifying the patients on the basis of the presence or absence of the well-established *NOD2/CARD15* mutations, we looked for susceptibility factors being present in one specific phenotypic subpopulations.

MATERIALS AND METHODS

Study population

We studied a cohort of 205 Caucasian unrelated consecutive patients with CD who were recruited in a Unit of Inflammatory Bowel Disease (IBD) from a single tertiary referral center in Madrid, Spain. Diagnosis of Crohn's disease was based on standard clinical, radiologic, endoscopic, and histologic criteria[12]. Phenotypic details were obtained by review of clinical charts and personal interview with the patients. The same clinical questionnaire was completed for each patient. This questionnaire included: date of birth, sex, familial IBD, age at diagnosis, follow-up interval, smoking habits, history of surgery (tonsillectomy, appendectomy), definitions of the Vienna classification for age at diagnosis (A1, < 40 years; A2, ⩾ 40 years), disease location (L1, terminal ileum; L2, colon; L3, ileocolon; L4, upper gastrointestinal), behavior (B1, nonstricturing nonpenetrating; B2, stricturing; B3, penetrating), perianal disease (defined as the presence of perianal abscess, fistulas and/or ulceration), extraintestinal clinical manifestations (articular and cutaneous), and previous treatment as an indication of severity of disease (surgical intervention, corticosteroids, immunosuppressant agents, infliximab). All patient data were recorded by a gastroenterologist from the Unit of IBD (J. L. M.) who was blind to the genotype status of each patient. The protocol was approved by the Ethics Committee of the Hospital Clínico San Carlos, Madrid, and all patients were included in the study after giving informed consent.

Genotyping

IL-10 polymorphisms: IL-10G and IL-10R microsatellites were amplified using primers and conditions as previously described[13]. Blood samples were subsequently denatured and run on an ABI Prism 3100 automatic sequencer (Applied Biosystems, Foster City, CA, USA). Each sample included an internal size standard (HD400 ROX, Applied Biosystems) in order to achieve a highly consistent measure. The results were analyzed using GeneMapper v3.0 (Applied Biosystems).

As previously described[14], a combined amplification of the IL-10G microsatellite and the -1082 and -819 SNPs was performed. Our typing method allowed us to construct haplotypes directly. SNPs at positions -1082, -819 and -592 only form three different haplotypes in our population[15,16], namely, ACC, ATA and GCC. Based on this previous finding, we only typed the samples for the two first SNPs, as the information provided by the third one is redundant.

NOD2/CARD15 polymorphisms: SNP13 (Leu1007fsinsC) was genotyped using a TaqMan assay (Applied Biosystems, Foster City, CA, USA). Primers and probes used were as previously described[5, 17] and PCR products were analysed on an ABI 7700 Sequence Detector (Applied Biosystems). SNP8 (Arg702Trp) (sense, 5´-CAT CTG AGA AGG CCC TGC TC(C/T)-3´; antisense, 5´-CAG ACA CCA GCG GGC ACA-3´) and SNP12 (Gly908Arg) (sense, 5´-TTG GCC TTT TCA GAT TCT GG (G/C)-3´; antisense, 5´-CCC CTC GTC ACC CAC TCT G-3´) were typed by allele-specific PCR. Detection of wild-type/mutant variants was assessed in an ABI 7700 Sequence Detector by an SYBRGreen assay. Previously sequenced samples were used as controls. In cases of doubt, samples were sequenced to confirm the result.

Statistical analysis

The frequencies for the IL-10 polymorphisms and *NOD2/CARD15* mutations were estimated by counting gene and calculating sample proportions. Subsequently, Hardy Weinberg equilibrium for each of the polymorphisms was tested to check for Mendelian inheritance using χ^2 test with one degree of freedom. Carrier status was considered if any subject inherited at least one copy of the mutant allele 2. The association between IL-10 polymorphisms and phenotypic characteristics of CD was estimatied by the relative risk (*RR*) with the 95% confidence interval (*CI*). Logistic regression analysis was perfomed to assess whether IL-10 polymorphisms were correlated with a particular clinical phenotype. The multiple logistic regression analysis was

Table 1 Distribution of IL-10G14 microsatellite allele and -1082G allele stratified by NOD2/CARD15 status

Allele frequencies	At least one CARD15/NOD2 mutation positive *n* = 76 (%)	CARD15/NOD2 mutation negative *n* = 129 (%)	*P*
IL-10G14 (*n* = 47)	22 (28.9)	25 (19.4)	0.115
-1082G (*n* = 146)	54 (71.1)	92 (71.3)	0.96
IL-10G14+-1082G (*n* = 29)	13 (17.1)	16 (12.4)	0.35

Table 2 Distribution of -1082G allele among different clinical subgroups of CD stratified by NOD2/CARD15 status

Phenotypic characteristics	Phenotype frequency of -1082 IL-10G(+) (*n* = 146) (%)	*P*	CARD15/NOD2(+) -1082 IL-10 G(+) (*n* = 54) (%)	*P*	CARD15/NOD2(-) -1082 IL-10 G(+) (*n* = 92) (%)	*P*
Sex						
Men	67 (45.9)		24 (44.4)	0.77	43 (46.7)	0.45
Women	79 (54.1)	0.67	30 (55.5)		49 (53.3)	
Age at diagnosis (yr)						
A1, < 40	117 (80.1)		46 (85.2)	0.5	71 (77.2)	0.41
A2, ⩾40	29 (19.9)	0.72	8 (14.8)		21 (22.8)	
Family history	27 (18.5)	0.43	10 (18.5)	0.43	17 (18.5)	0.76
Smokers	64 (43.8)	0.55	24 (44.4)	0.86	40 (43.5)	0.65
Appendectomy	20 (13.7)	0.55	7 (12.9)	0.93	13 (14.1)	0.49
Tonsillectomy	22 (15.1)	0.11	5 (9.3) 0.66		17 (18.5)	0.19
Disease behavior						
Nonstricturing, nonpenetrating (B1)	63 (43.2)	0.87	24 (44.4)	0.58	39 (42.4)	0.86
Stricturing (B2)	22 (15.1)		9 (16.7)		13 (14.1)	
Penetrating (B3)	61 (41.8)		21 (38.9)		40 (43.5)	
Location of disease						
Terminal ileum (L1)	70 (47.9)	0.63	29 (53.7)	0.021	41 (44.6)	0.43
Colon (L2)	24 (16.4)		5 (9.3)		19 (20.7)	
Ileocolon (L3)	47 (32.2)		19 (35.2)		28 (30.4)	
Upper gastrointestinal (L4)	5 (3.4)		1 (1.9)		4 (4.3)	
Perianal	39 (26.7)	0.30	11 (20.4)	1	28 (30.4)	0.3
Extraintestinal clinical manifestations						
Cutaneous	27 (18.5)	0.56	9 (16.7)	0.29	18 (19.6)	0.93
Articular	49 (33.6)	0.69	16 (29.6)	0.83	33 (35.9)	0.83
Treatment						
Surgical intervention	61 (41.8)	0.76	22 (40.7)	0.71	39 (42.4)	0.92
Infliximab	19 (13.0)	0.06	5 (9.3) 0.032		14 (15.2)	0.61
Immunosuppressants	63 (43.2)	0.81	25 (46.3)	0.72	38 (41.3)	0.63

[1] -1082G allele in NOD2/CARD15 mutation positive patients: ileocolonic location ($P = 0.008$, $RR = 1.52$, 95% $CI = 1.21$-1.91). [2] -1082G allele in NOD2/CARD15 mutation positive patients: infliximab ($P = 0.03$, $RR = 0.54$, 95%$CI = 0.27$-1)

adjusted for age (years). A two-tailed *P* value equal to or less than 0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 10.07 for Windows (SPSS Inc., Chicago, Ill. USA).

RESULTS

The cohort of 205 patients with Crohn's disease consisted of 109 men and 96 women. The median age at diagnosis was 27 years (mean 31.6, range 8-80) with an interquartile range of 22-26 years. The median duration of follow-up was 11 years (mean 12.57, range 3-47) with an interquartile range of 7-16 years.

No statistically significant difference in IL-10G14 and -1082G or both allele frequencies was found between *NOD2/CARD15* mutation positive and negative patients (Table 1).

The genotype-phenotype correlations are shown in Tables 2 and 3. No Vienna classifications of the disease, neither risk factors, clinical manifestations, or treatment modalites were associated with either IL-10G14 or -1082G alleles. A gene-dosage effect (IL-10G14 and -1082G alleles) on phenotypic characteristics was not observed (data not shown).

When we examined the associated IL-10G14 and -1082G alleles in the *NOD2/CARD15* mutation positive and negative patients separately, three new positive associations were found. With regard to Vienna classifications of the disease, ileocolonic disease was significantly associated with -1082G allele in the *NOD2/CARD15* mutation positive patients ($P = 0.008$, Table 2). On the other hand, relative to risk factors for Crohn disease, two significant associations of the IL-10G14 allele carriership and history of appendectomy ($P = 0.002$) and smoking habit at diagnosis ($P = 0.02$) in the *NOD2/CARD15* mutation positive patients as compared with the negative patients were observed (Table 3). The multivariate analysis demonstrated that IL-10G14 allele was associated with history of appendectomy ($P = 0.001$, $RR = 2.15$, 95%$CI = 1.1$-4.30) and with smoking habit at diagnosis ($P = 0.04$, $RR = 1.29$, 95%$CI = 1.04$-4.3).

DISCUSSION

In this study, we performed a genotype-phenotype correlation study in a cohort of 205 Caucasian patients with Crohn's disease from the community of Madrid

Table 3 Distribution of IL-10G14 allele among the different clinical subgroups of CD stratified by NOD2/CARD15 status

Phenotypic characteristics	Phenotype frequency of IL-10.G14 (+) (*n* = 47) (%)	*P*	CARD15/NOD2 (+) IL-10.G14, (*n* = 22) (%),	*P*	CARD15/NOD2 (-) IL-10.G14, (*n* = 25) (%),	*P*
Sex						
Men	23 (48.9)	0.74	13 (59.1)	0.08	10 (40)	0.32
Women	24 (51.1)		9 (40.9)		15 (60)	
Age at diagnosis (yr)						
A1, < 40	39 (83.0)	0.5	21 (95.5)	0.27	18 (72)	0.68
A2, ≥40	8 (17.0)		1 (4.5)		7 (28)	
Family history	9 (19.1)	0.54	4 (18.2)	0.52	5 (20)	0.88
Smokers	24 (51.1)	0.51	16 (72.7)	0.02[1]	8 (32)	0.41
Appendectomy	9 (19.1)	0.32	6 (27.3)	0.002[2]	3 (12)	0.59
Tonsillectomy	5 (10.6)	0.63	3 (13.6)	0.48	2 (8)	0.36
Disease behavior						
Nonstricturing, nonpenetrating (B1)	19 (40.4)	0.61	6 (27.3)	0.19	13 (52)	0.62
Stricturing (B2)	9 (19.1)		6 (27.3)		3 (12)	
Penetrating (B3)	19 (40.4)		10 (45.5)		9 (36)	
Location of disease						
Terminal ileum (L1)	23 (48.9)	0.26	15 (68.2)	0.54	8 (32)	0.24
Colon (L2)	4 (8.5)		1 (4.5)		3 (12)	
Ileocolon (L3)	17 (36.2)		5 (22.7)		12 (48)	
Upper gastrointestinal (L4)	3 (6.4)		1 (4.5)		2 (8)	
Perianal	14 (29.8)	0.86	4 (18.2)	0.47	10 (40)	0.28
Extraintestinal clinical manifestations						
Cutaneous	8 (17.0)	0.62	3 (13.6)	0.30	5 (20)	0.56
Articular	13 (27.7)	0.32	6 (27.3)	0.53	7 (28)	0.36
Treatment						
Surgical intervention	20 (42.6)	0.98	10 (45.5)	0.8	10 (40)	0.82
Infliximab	7 (14.9)	0.79	5 (22.7)	0.31	2 (8)	0.21
Immunosuppressants	18 (38.3)	0.61	10 (45.5)	0.81	8 (32)	0.23

[1]IL-10G14 in NOD2/CARD2 mutation positive patients: smokers *vs* non-smokers ($P = 0.02$, $RR = 2.47$, 95%$CI = 1.28$-4.8). [2]IL-10 G14 in NOD2/CARD2 mutation positive patients: appendectomy *vs* non-appendectomy ($P = 0.002$, $RR = 3.29$, 95%$CI = 1.45$-7)

(central Spain) who had been followed-up for a mean of 12.57 years. The clinical diagnosis of Crohn's disease was confirmed by the criteria of Gasche *et al*[18]. Our results showed that a relation existed between disease location (ileocolon), risk factors for CD (appendicectomy and smoking habit) and genetic heterogeneity in our population. This could suggest an epistatic interaction of both genes.

CD is an extensively heterogeneous disease. Epidemiologic and genetic data suggest that heterogeneity of CD may be genetically determined. Recently, Ahmad *et al*[19] have shown the importance of the *NOD2/CARD15* gene and the HLA region in determining clinical subgroups of CD. Similarly, in our CD population, we confirmed the association between *NOD2/CARD15* mutations and ileal disease and the strong association between DRB1*0103 allele and colonic disease[17]. These studies may provide an initial basis for the construction of a molecular classification of CD[20].

Location of disease is the variable that remains more stable during the course of the disease[21] and it showed a stronger association with mutations of the *NOD2/CARD15* gene in genotype-phenotype studies using the Vienna classification. In our population[7], like others[19,22,23], possession of an *NOD2/CARD15* variant was significantly associated with ileal disease. On the contrary, mutations of the *NOD2/CARD15* gene were exceptional in patients with solely colonic involvement[7]. In contrast to findings in the Norwegian and German populations[24] and similar to findings in the British population[19,25], we could not find an association between ileocolonic location and mutations of the *NOD2/CARD15* gene[7]. In contrast, this association has been found between the *NOD2/CARD15* variants and IL-10 -1082G carriers. This suggests the importance of classifying the patients according to the different genes implicated in the etiopathogenesis of CD and, therefore, of performing the molecular characterization of CD patients. Tagore *et al*[26] have shown that IL-10 production is associted with three biallelic polymorphisms within the promotor region of the IL-10. The allele -1082G is associated with higher IL-10 production in peripheral blood leukocytes. This different levels of IL-10 expression could explain the diverse phenotypic clinical behaviour of CD in specific genetic susceptibility background, like *NOD2/CARD15*.

No other factor is likely to contribute in isolation to the pathogenesis of CD. The modulation of the immune system either locally or systemically has an important role to play in the etiology and pathogenesis of this disease. The IL-10 gene has special interest for a candidate gene approach in CD. The biology of IL-10 is highly complex: a potent down-regulator of CD peripheral monocyte and intestinal lamina propria mononuclear cells activator *in vitro* and *in vivo*[27]. Both human IL-10 and murine IL-10 exert immunostimulatory effects by up-regulating MHC class II expression in B lymphocytes and inducing cytotoxic T-cell differentiation[28]. Due to the dual regulatory function of IL-10 itself, its gene is indeed an interesting susceptibility candidate and it would be worthwhile to know whether environment factors could modulate the IL-10 function.

The IL-10 gene knockout mouse spontaneously develops a chronic enterocolitis[29] and gene therapy using an adenovirus IL-10 contruct is successful in preventing experimental colitis in rats[30]. Moreover, there have been preliminary reports of amelioration of clinical symptoms of CD following administration of human recombinant IL-10[27,31].

Regarding the risk factors for CD, we found two significant associations between carriership of the IL-10G14 microsatellite allele in CARD15-positive patients and previous history of appendectomy and smoking habit at diagnosis. The interaction between genetics and smoking has been demonstrated in siblings from mixed-disease families, where some individuals develop CD and others in the same family develop ulcerative colitis. There is a strong positive relationship between smoking and CD and an equally strong negative relationship between smoking and ulcerative colitis[32]. Appendectomy provides a spectrum of protection against ulcerative colitis development and progression, whereas its role in CD remains unclear. Russel *et al*[33] have also noted a positive association of CD and previous appendectomy, suggesting that, in some cases, appendectomy is a result of still undiagnosed CD. Recently, other retrospective study concluded the risk of CD after appendectomy was associated with an increased risk of CD dependent on the patient´s sex, age, and the diagnosis at operation[34]. Future work should pursue to investigate the epidemiological relationships in CD, addressing a greater number of potentially important confounders, such as smoking, hygiene, and pathology of the appendix. And parallel with these clinical observations, new target could be defined by genetic and immunologic analysis to evaluate if appendicitis could be correlated with any particular genetic modification involved for patients with CD[35].

In conclusion, our study has shown that in the Spanish population from Madrid, in CD patients carrying at least an *NOD2/CARD15* mutant, the -1082G allele might be associated with ileocolonic disease, and the IL-10G14 microsatellite allele might be associated with previous history of appendectomy and smoking habit at diagnosis. Identification of plausible factors that may interact with genes is a promising step toward understanding how sequence variation influences disease susceptibility.

REFERENCES

1 **Shanahan F**. Crohn's disease. *Lancet* 2002; **359**: 62-69
2 **Morahan G**, Morel L. Genetics of autoimmune diseases in humans and in animal models. *Curr Opin Immunol* 2002; **14**: 803-811
3 **Fiocchi C**. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 1998; **115**: 182-205
4 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599-603
5 **Hampe J**, Cuthbert A, Croucher PJ, Mirza MM, Mascheretti S, Fisher S, Frenzel H, King K, Hasselmeyer A, MacPherson AJ, Bridger S, van Deventer S, Forbes A, Nikolaus S, Lennard-Jones JE, Foelsch UR, Krawczak M, Lewis C, Schreiber S, Mathew CG. Association between insertion mutation in NOD2 gene and Crohn's disease in German and British populations. *Lancet* 2001; **357**: 1925-1928
6 **Ogura Y**, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nuñez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606
7 **Mendoza JL**, Murillo LS, Fernández L, Peña AS, Lana R, Urcelay E, Cruz-Santamaría DM, de la Concha EG, Díaz-Rubio M, García-Paredes J. Prevalence of mutations of the NOD2/CARD15 gene and relation to phenotype in Spanish patients with Crohn disease. *Scand J Gastroenterol* 2003; **38**: 1235-1240
8 **van der Linde K**, Boor PP, Sandkuijl LA, Meijssen MA, Savelkoul HF, Wilson JH, de Rooij FW. A Gly15Arg mutation in the interleukin-10 gene reduces secretion of interleukin-10 in Crohn disease. *Scand J Gastroenterol* 2003; **38**: 611-617
9 **Mocellin S**, Panelli MC, Wang E, Nagorsen D, Marincola FM. The dual role of IL-10. *Trends Immunol* 2003; **24**: 36-43
10 **Fernandez L**, Martinez A, Mendoza JL, Urcelay E, Fernandez-Arquero M, Garcia-Paredes J, Diaz-Rubio M, de la Concha EG. Interleukin-10 polymorphisms in Spanish patients with IBD. *Inflamm Bowel Dis* 2005; **11**: 739-743
11 **Netea MG**, Kullberg BJ, de Jong DJ, Franke B, Sprong T, Naber TH, Drenth JP, Van der Meer JW. NOD2 mediates anti-inflammatory signals induced by TLR2 ligands: implications for Crohn's disease. *Eur J Immunol* 2004; **34**: 2052-2059
12 **Lennard-Jones JE**. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989; **170**: 2-6; discussion 16-9
13 **Eskdale J**, Gallagher G, Verweij CL, Keijsers V, Westendorp RG, Huizinga TW. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. *Proc Natl Acad Sci U S A* 1998; **95**: 9465-9470
14 **Cavet J**, Middleton PG, Segall M, Noreen H, Davies SM, Dickinson AM. Recipient tumor necrosis factor-alpha and interleukin-10 gene polymorphisms associate with early mortality and acute graft-versus-host disease severity in HLA-matched sibling bone marrow transplants. *Blood* 1999; **94**: 3941-3946
15 **Martinez A**, Pascual M, Pascual-Salcedo D, Balsa A, Martin J, de la Concha EG. Genetic polymorphisms in Spanish rheumatoid arthritis patients: an association and linkage study. *Genes Immun* 2003; **4**: 117-121
16 **Martinez Doncel A**, Rubio A, Arroyo R, de las Heras V, Martín C, Fernandez-Arquero M, de la Concha EG. Interleukin-10 polymorphisms in Spanish multiple sclerosis patients. *J Neuroimmunol* 2002; **131**: 168-172
17 **Fernandez L**, Mendoza JL, Martinez A, Urcelay E, Fernandez-Arquero M, Garcia-Paredes J, Peña AS, Diaz-Rubio M, de la Concha EG. IBD1 and IBD3 determine location of Crohn's disease in the Spanish population. *Inflamm Bowel Dis* 2004; **10**: 715-722
18 **Gasche C**, Scholmerich J, Brynskov J, D'Haens G, Hanauer SB, Irvine EJ, Jewell DP, Rachmilewitz D, Sachar DB, Sandborn WJ, Sutherland LR. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. *Inflamm Bowel Dis* 2000; **6**: 8-15
19 **Ahmad T**, Armuzzi A, Bunce M, Mulcahy-Hawes K, Marshall SE, Orchard TR, Crawshaw J, Large O, de Silva A, Cook JT, Barnardo M, Cullen S, Welsh KI, Jewell DP. The molecular classification of the clinical manifestations of Crohn's disease. *Gastroenterology* 2002; **122**: 854-866
20 **Bell J**. The new genetics in clinical practice. *BMJ* 1998; **316**: 618-620
21 **Louis E**, Collard A, Oger AF, Degroote E, Aboul Nasr El Yafi FA, Belaiche J. Behaviour of Crohn's disease according to the Vienna classification: changing pattern over the course of the disease. *Gut* 2001; **49**: 777-782
22 **Lesage S**, Zouali H, Cézard JP, Colombel JF, Belaiche J, Almer S, Tysk C, O'Morain C, Gassull M, Binder V, Finkel Y, Modigliani R, Gower-Rousseau C, Macry J, Merlin F, Chamaillard M, Jannot AS, Thomas G, Hugot JP. CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 2002; **70**: 845-857

23 **Vavassori P**, Borgiani P, D'Apice MR, De Negris F, Del Vecchio Blanco G, Monteleone I, Biancone L, Novelli G, Pallone E. 3020insC mutation within the NOD2 gene in Crohn's disease: frequency and association with clinical pattern in an Italian population. *Dig Liver Dis* 2002; **34**: 153
24 **Hampe J**, Grebe J, Nikolaus S, Solberg C, Croucher PJ, Mascheretti S, Jahnsen J, Moum B, Klump B, Krawczak M, Mirza MM, Foelsch UR, Vatn M, Schreiber S. Association of NOD2 (CARD 15) genotype with clinical course of Crohn's disease: a cohort study. *Lancet* 2002; **359**: 1661-1665
25 **Cuthbert AP**, Fisher SA, Mirza MM, King K, Hampe J, Croucher PJ, Mascheretti S, Sanderson J, Forbes A, Mansfield J, Schreiber S, Lewis CM, Mathew CG. The contribution of NOD2 gene mutations to the risk and site of disease in inflammatory bowel disease. *Gastroenterology* 2002; **122**: 867-874
26 **Tagore A**, Gonsalkorale WM, Pravica V, Hajeer AH, McMahon R, Whorwell PJ, Sinnott PJ, Hutchinson IV. Interleukin-10 (IL-10) genotypes in inflammatory bowel disease. *Tissue Antigens* 1999; **54**: 386-390
27 **Schreiber S**, Heinig T, Thiele HG, Raedler A. Immunoregulatory role of interleukin 10 in patients with inflammatory bowel disease. *Gastroenterology* 1995; **108**: 1434-1444
28 **Li MC**, He SH. IL-10 and its related cytokines for treatment of inflammatory bowel disease. *World J Gastroenterol* 2004; **10**: 620-625
29 **Kühn R**, Löhler J, Rennick D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 1993; **75**: 263-274
30 **Barbara G**, Xing Z, Hogaboam CM, Gauldie J, Collins SM. Interleukin 10 gene transfer prevents experimental colitis in rats. *Gut* 2000; **46**: 344-349
31 **van Deventer SJ**, Elson CO, Fedorak RN. Multiple doses of intravenous interleukin 10 in steroid-refractory Crohn's disease. Crohn's Disease Study Group. *Gastroenterology* 1997; **113**: 383-389
32 **Bridger S**, Lee JC, Bjarnason I, Jones JE, Macpherson AJ. In siblings with similar genetic susceptibility for inflammatory bowel disease, smokers tend to develop Crohn's disease and non-smokers develop ulcerative colitis. *Gut* 2002; **51**: 21-25
33 **Russel MG**, Dorant E, Brummer RJ, van de Kruijs MA, Muris JW, Bergers JM, Goedhard J, Stockbrügger RW. Appendectomy and the risk of developing ulcerative colitis or Crohn's disease: results of a large case-control study. South Limburg Inflammatory Bowel Disease Study Group. *Gastroenterology* 1997; **113**: 377-382
34 **Andersson RE**, Olaison G, Tysk C, Ekbom A. Appendectomy is followed by increased risk of Crohn's disease. *Gastroenterology* 2003; **124**: 40-46
35 **Adani GL**, Baccarani U, Risaliti A, Donini A, Aoki T, Avital I. Appendicectomy and Crohn's disease: clinical and genetic associations. *Gastroenterology* 2003; **125**: 1562-1563

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RAPID COMMUNICATION

Atrial fibrillation after surgery for esophageal carcinoma: Clinical and prognostic significance

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Abstract

AIM: To retrospectively evaluate the clinical relevance, perioperative risk factors, outcome of different pharmacological prophylaxis, and short-term prognostic value of atrial fibrillation (AF) after surgery for esophageal carcinoma.

METHODS: We retrospectively studied 63 patients with AF after surgery for esophageal carcinoma in comparison with 126 patients without AF after esophagectomy during the same time. Postoperative AF incidence was related to different clinical factors possibly involved in its occurrence and short-term survival.

RESULTS: A strong relationship was observed between AF and postoperative hypoxia, history of chronic obstructive pulmonary disease (COPD), postoperative thoracic–gastric dilatation, age older than 65 years, male sex and history of cardiac disease. No difference was observed between the two groups with regard to short-term mortality and length of hospital stay.

CONCLUSIONS: AF occurs more frequently after esophagectomy in aged and male patients. Other factors contributing to postoperative AF are history of COPD and cardiac disease, postoperative hypoxia and thoracic–gastric dilatation.

Key words: Esophageal carcinoma; Atrial fibrillation; Surgery

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INTRODUCTION

Atrial fibrillation (AF) is a frequently occurring arrhythmia after esophageal procedures. It has been suggested that AF may be generally related to a worse prognosis and a longer postoperative hospital stay[1]. Reports on the effectiveness of alternative strategies to control AF are contradictory[2,3]. Therefore identification of all high-risk populations will allow targeted use and hence more cost-effective and successful application of these methods can be achieved.

The purpose of the present study was to retrospectively evaluate the clinical relevance, perioperative risk factors, outcome of different pharmacological interventions, and short-term prognostic value of AF after surgery for esophageal carcinoma.

MATERIALS AND METHODS

Patients

From 1998 to the end of 2003, a total of 63 consecutive patients had AF after surgery for esophageal carcinoma in our institution. AF was defined as a sustained or repetitive electrocardiographically documented arrhythmia requiring antiarrhythmic therapy[4]. During the same time of surgery, 126 patients without postoperative AF after esophageal carcinoma resection were randomly chosen as the control group. A retrospective chart review was performed on all the patients with and without AF. Patients were identified by search of registry database and hospital medical records. Records were reviewed by two independent researchers to confirm the diagnosis of AF.

There were 63 AF patients, 55 males and 8 females with an average age of 64.7 (range, 51-83) years. The onset of AF occurred on the first postoperative day in 22 patients (34.9%), on the second postoperative day in 30 patients (47.6%), on the third postoperative day in 7 patients (11.1%), and on the fourth postoperative day, and later in 4 patients (6.4%). The control group consisted of 126 patients, 94 males, and 32 females with an average age of 57.2 (range, 32-79) years. Patients with a known history of atrial dysrhythmias or those receiving digoxin were excluded from analysis.

Methods

Postoperative AF incidence was related to different pre-

Table 1 Preoperative factors associated with postoperative AF (%)

Group	Age older than 65 years	Male sex	Cardiac diseases	COPD	Hypertension	Diabetes mellitus
AF	39.68 (25/63)[b]	87.30 (55/63)[a]	19.05 (12/63)[a]	46.03 (29/63)[b]	9.52 (6/63)	14.29 (9/63)
Control	21.43 (27/126)[b]	74.60 (94/126)	7.14 (9/126)	11.11 (14/126)	5.56 (7/126)	12.70 (16/126)

[a]$P<0.05$, [b]$P<0.01$ *vs* control group.

Table 2 Intraoperative and postoperative factors associated with postoperative AF (%)

Group	Right thorax approach	Anastomosis at the neck	Anastomosis below the aortic arch	Anastomosis above the aortic arch	Hypotension in operation	Postoperative fever	Postoperative hypoxia	Thoracic–gastric dilatation
AF	11.11 (7/63)	22.22 (14/63)	14.29 (9/63)	63.49 (40/63)	7.94 (5/63)	14.29 (9/63)	25.40 (16/63)[b]	31.75 (20/63)[b]
Control	7.94 (10/126)	11.90 (15/126)	10.32 (13/126)	77.78 (98/126)	7.14 (9/126)	8.73 (11/126)	4.76 (6/126)[b]	18.25 (23/126)[b]

[b]$P<0.01$ *vs* control group.

operative, intraoperative, and postoperative clinical factors possibly involved in its occurrence and short-term survival. The demographic data that were analyzed included patients' age, sex, history of cardiac disease, chronic obstructive pulmonary disease (COPD, defined as $FEV_1 \leqslant 70\%$ predicted and FEV_1/FVC ratio $\leqslant 70\%$ in pulmonary function tests), diabetes mellitus, and hypertension. Intraoperative and postoperative factors examined were surgical approach (via the left or the right thorax), site of anastomosis (at the neck, above or below the aortic arch), hypotension in operation (defined as systolic blood pressure lower than 6 kPa, persisting for more than 5 min), postoperative fever (defined as body temperature higher than 38.5 °C, persisting for more than 3 d), hypoxia (defined as aortic blood oxygen saturation lower than 93%, persisting for more than 1 h before the onset of AF), thoracic-gastric dilatation before the onset of AF confirmed by chest X-ray, 30-d mortality rate and length of hospital stay.

Statistical analysis

Statistical analysis was performed with the SPSS software version 10.0. Intergroup difference was determined using Student's t test and Fisher's exact test. χ^2 test was used to analyze categorical data. To identify which factors could predict AF, univariate, and stepwise multiple logistic regression analysis was used. $P<0.05$ was considered statistically significant.

RESULTS

A higher incidence of AF was found in male patients older than 65 years, with a history of cardiac diseases and COPD than that in the control group patients (Table 1). There was no significant difference in patients with a history of hypertension and diabetes mellitus between the two groups. AF patients had a higher incidence of postoperative hypoxia and thoracic-gastric dilatation than the control group (Table 2). There was no significant difference in surgical approach, anastomosis site, intraoperative hypotension and postoperative fever between the two groups.

Results from the univariate analysis for the association of each factor with AF are summarized in Table 3. The factors associated with increased risk for AF on multivariate analysis included postoperative hypoxia, history of COPD, thoracic-gastric dilatation, age older than 65 years, male sex and history of cardiac disease. The incidence of AF was not dependent on the history of hypertension and diabetes mellitus, intraoperative hypotension, surgical approach, anastomosis site, and postoperative fever.

In the AF group, 15 patients who did not receive any antiarrhythmic drug therapy in the early stage of AF were given oxygen and sedation therapy. Only one of the 15 patients (6.67%) had sinus rhythm spontaneously within 24 h. All the other 62 patients were treated with antiarrhythmic drugs such as cedilanid, isoptin, propafenone and amiodarone. It was considered successful if the heart rhythm changed to sinus rhythm in 24 h after the treatment. Otherwise, other antiarrhythmic drugs were used. The outcome of antiarrhythmic therapy is presented in Table 4.

Death within 30 d after the surgery occurred in 2 AF patients with a mortality of 3.2% (2/63), and in 2 patients of the control group with a mortality of 1.6% (2/126) ($P>0.05$). The overall mortality in our patients was 2.1% (4/189). None of these deaths were directly attributed to AF. The average length of hospital stay was 10.65 ± 0.87 d for the AF group and 9.98 ± 0.96 d for the control group ($P>0.05$).

DISCUSSION

AF after esophagectomy remains one of the most frequent complications. The cause for postoperative AF is unclear. However, previous studies have shown different results, sometimes contradictory with regard to the clinical significance of various factors for AF after esophagectomy[1,5]. We found that postoperative hypoxia, history of COPD, thoracic-gastric dilatation, age older than 65 years, male sex, and history of cardiac disease were predictors of postoperative AF. We considered that only clinically significant

Table 3 Univariate analysis of risk factors for postoperative AF

Risk factors	*P*
Postoperative hypoxia	<0.001
COPD	0.001
Thoracic-gastric dilatation	0.009
Age more than 65 y	0.009
Male sex	0.017
History of heart disease	0.038
Postoperative fever	0.051
History of hypertension	0.062
Surgical approach	0.143
Anastomosis site	0.189
History of diabetes mellitus	0.412
Intraoperative hypotension	0.475

Table 4 Outcome of AF after drug treatment

Prophylaxis	Cases	Successful antiarrhythmic cases (%)
Cedilanid	51	18 (35.29)
Isoptin	17	2 (11.76)
Propafenone	21	11 (52.38)
Amiodarone	20	20 (100.00)

AF defined by electrocardiography had an association with hemodynamic changes. A possible limitation of our study is that continuous electrocardiographic monitoring was not employed to detect asymptomatic AF, as our study was designed to evaluate clinically significant AF, which was the focus of patients' complaints and needed therapeutic interventions. Our conclusions, therefore, do not apply to the occurrence of asymptomatic AF after the surgery for esophageal carcinoma.

We did not find differences in survival rate and length of hospital stay between patients who developed AF and those who did not in the early postoperative period. We found that some pharmacological interventions were effective for AF after esophagectomy, but symptomatic management without drug treatment was not effective, suggesting that AF, if treated promptly, is fairly tolerated in all the patients. The peak incidence of AF on the second day after surgery, with more than 80% of events occurring during the first three postoperative days, is consistent with the findings of a previous study[6]. We speculate that AF after esophagectomy is precipitated by the resolution of inflammatory response following blunt or sharp surgical trauma to sympathovagal nerve fibers supplying the heart for 1-4 d postoperatively, which alter the autonomic modulation of atrial myocardial cells to endogenous catecholamines.

The identification of reliable predictors of AF after esophagectomy may help us to develop corresponding preventive strategies. The presence of postoperative hypoxia and history of COPD were associated with the development of AF in our study. It has been well established that due to diminished cardiopulmonary reserve, patients with COPD and hypoxia are more prone to perioperative complications following noncardiac thoracic surgical procedures in general[7,8]. The definition of COPD might vary between different authors and our definition was based on the pulmonary function testing, and some authors' definition is based on the presence or absence of bronchodilator treatment[4].

The association between postoperative thoracic-gastric dilatation and AF found in our analysis has not been previously demonstrated. We speculate that it may correlate with hypoxia, physical influence on the heart from the dilated stomach, and the uncomfortable feeling of patients for the thoracic-gastric dilation. It is not surprising that advanced age is a strong predictor of postoperative AF, because at the age of 75, only approximately 10% of normal sinus node pacemaker cells remain[9]. It is well known that age-related cardiac structural changes, such as increased fibrous and adipose tissue in the sinoatrial node, and focal interstitial deposits of amyloid in the atria, and atrial dilations may play a significant role in the genesis of arrhythmia[10]. The age is closely related with AF[11].

The association between male sex and AF might be explained by the effect of sex on immune response. One hypothesis for the cause of postoperative AF is atrial or pulmonary vein inflammation[12]. After trauma, male patients have an increased proinflammatory immune response compared to female patients[13], and this might lead to increased AF after surgical trauma. The association of cardiac disease with AF has been demonstrated inconsistently by other investigators[14]. The common occurrence of AF after cardiac surgery supports the association of cardiac disease with AF after thoracic surgery. The lack of association between hypertension and AF in our analysis is also noteworthy. Other investigators have shown this association, but their analysis is limited to patients with lung cancer[15].

In conclusion, AF is associated with postoperative hypoxia, history of COPD, thoracic-gastric dilatation, age older than 65 years, male sex, and history of cardiac disease. The identification of patients with a high risk for AF will allow more direct application of pharmacological and alternative methods of prophylaxis.

REFERENCES

1 **Amar D**, Roistacher N, Burt M, Reinsel RA, Ginsberg RJ, Wilson RS. Clinical and echocardiographic correlates of symptomatic tachydysrhythmias after noncardiac thoracic surgery. *Chest* 1995; **108**: 349-354

2 **Van Mieghem W**, Tits G, Demuynck K, Lacquet L, Deneffe G, Tjandra-Maga T, Demedts M. Verapamil as prophylactic treatment for atrial fibrillation after lung operations. *Ann Thorac Surg* 1996; **61**: 1083-1085; discussion 1086

3 **Van Mieghem W**, Coolen L, Malysse I, Lacquet LM, Deneffe GJ, Demedts MG. Amiodarone and the development of ARDS after lung surgery. *Chest* 1994; **105**: 1642-1645

4 **Vaporciyan AA**, Correa AM, Rice DC, Roth JA, Smythe WR, Swisher SG, Walsh GL, Putnam JB. Risk factors associated with atrial fibrillation after noncardiac thoracic surgery: analysis of 2588 patients. *J Thorac Cardiovasc Surg* 2004; **127**: 779-786

5 **Asamura H**, Naruke T, Tsuchiya R, Goya T, Kondo H, Suemasu K. What are the risk factors for arrhythmias after thoracic operations? A retrospective multivariate analysis of 267 consecutive thoracic operations. *J Thorac Cardiovasc Surg* 1993; **106**: 1104-1110

6 **Amar D**, Burt ME, Bains MS, Leung DH. Symptomatic

tachydysrhythmias after esophagectomy: incidence and outcome measures. *Ann Thorac Surg* 1996; **61**: 1506-1509
7 **Sekine Y**, Kesler KA, Behnia M, Brooks-Brunn J, Sekine E, Brown JW. COPD may increase the incidence of refractory supraventricular arrhythmias following pulmonary resection for non-small cell lung cancer. *Chest* 2001; **120**: 1783-1790
8 **Nielsen TD**, Bahnson T, Davis RD, Palmer SM. Atrial fibrillation after pulmonary transplant. *Chest* 2004; **126**: 496-500
9 **Wei JY**. Age and the cardiovascular system. *N Engl J Med* 1992; **327**: 1735-1739
10 **Lie JT**, Hammond PI. Pathology of the senescent heart: anatomic observations on 237 autopsy studies of patients 90 to 105 years old. *Mayo Clin Proc* 1988; **63**: 552-564
11 **Amar D**, Zhang H, Leung DH, Roistacher N, Kadish AH. Older age is the strongest predictor of postoperative atrial fibrillation. *Anesthesiology* 2002; **96**: 352-356
12 **Chung MK**, Martin DO, Sprecher D, Wazni O, Kanderian A, Carnes CA, Bauer JA, Tchou PJ, Niebauer MJ, Natale A, Van Wagoner DR. C-reactive protein elevation in patients with atrial arrhythmias: inflammatory mechanisms and persistence of atrial fibrillation. *Circulation* 2001; **104**: 2886-2891
13 **Angele MK**, Schwacha MG, Ayala A, Chaudry IH. Effect of gender and sex hormones on immune responses following shock. *Shock* 2000; **14**: 81-90
14 **Polanczyk CA**, Goldman L, Marcantonio ER, Orav EJ, Lee TH. Supraventricular arrhythmia in patients having noncardiac surgery: clinical correlates and effect on length of stay. *Ann Intern Med* 1998; **129**: 279-285
15 **Cardinale D**, Martinoni A, Cipolla CM, Civelli M, Lamantia G, Fiorentini C, Mezzetti M. Atrial fibrillation after operation for lung cancer: clinical and prognostic significance. *Ann Thorac Surg* 1999; **68**: 1827-1831

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RAPID COMMUNICATION

Expression of dendritic cell-specific intercellular adhesion molecule 3 grabbing nonintegrin on dendritic cells generated from human peripheral blood monocytes

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Abstract

AIM: To generate dendritic cells (DCs) from human peripheral blood and to detect the expression of dendritic cell-specific intercellular adhesion molecule 3 grabbing nonintegrin (DC-SIGN; CD209) for the further study of DC-SIGN in hepatitis C virus (HCV) transmission.

METHODS: Peripheral blood monocytes were isolated from blood of healthy individuals by Ficoll—Hypaque sedimentation and cultured in complete medium containing rhGM-CSF and rhIL-4. Cells were cultured for seven days, with cytokine addition every two days to obtain immature DCs. Characteristics of the cultured cells were observed under light and scanning microscope, and the expression of DC-SIGN was detected by immunofluorescence staining.

RESULTS: After seven-day culture, a large number of cells with typical characteristics of DCs appeared. Their characteristics were observed under light and scanning electron microscope. These cells had a variety of cell shapes such as those of bipolar elongate cells, elaborate stellate cells and DCs. DC-SIGN was detected by immunofluorescence staining and its expression level on cultivated dendritic cells was high.

CONCLUSION: DCs with a high expression of DC-SIGN can be generated from human peripheral blood monocytes in complete medium containing rhGM-CSF and rhIL-4.

Key words: Expression of DC-SIGN; Dentric cells; Peripheral blood monocytes.

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INTRODUCTION

Dendritic cells (DCs) are professional antigen presenting cells (APCs) critically involved in the initiation of T cell-dependent immune responses as a consequence of their high expression of MHC and co-stimulatory molecules. However, it is becoming increasingly clear that some pathogens subvert DC functions to escape immune surveillance[1]. Many cell-surface molecules of DCs can bind to viral envelope glycoproteins without mediating entry. Instead, they serve as capture receptors that disseminate viral particles to target organs or susceptible cells. The C type lectin of DC-SIGN, one of the cell-surface molecules of DCs, functions as a capture receptor for several viruses, such as HIV type 1 (HIV-1)[2], hepatitis C virus (HCV)[3] and dengue virus[4]. Therefore, further investigations of the function of DCs and the interactions between DC-SIGN and pathogens are helpful in determining their importance *in vivo*. In this study, we generated dendritic cells from peripheral blood mononuclear cells (PBMC) from normal donors and high DC-SIGN expression was detected on the surface of dendritic cells by immunofluorescence staining.

MATERIALS AND METHODS

Medium and reagents

RPMI1640 and fetal calf serum (FCS) were purchased from Gibco Laboratories (Grand Island, NY). FCS was heat-inactivated at 56℃ for 30 min. Human recombinant IL-4(rhIL-4) and rhGM-CSF were from R&D Systems (Abingdon, UK) and used at 1000U/mL, respectively. Monoclonal anti-human DC-SIGN phycoerythrin (FAB161P) was also from R&D Systems. Bovine serum albumin (BSA) was from Huamei Co. Human peripheral

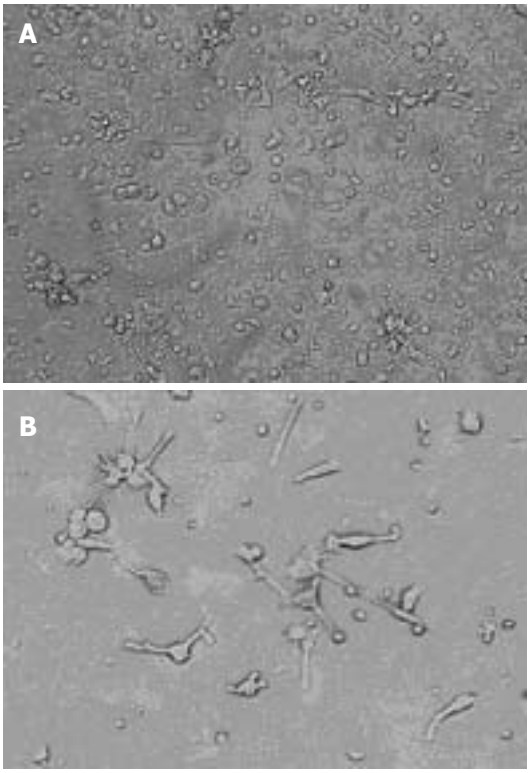

Figure 1 Dendritic cells in liquid cultures of complete RPMI 1640 supplemented with FCS, rhGM-CSF and rhIL-4. **A**: Low-power view of adherent PBMC after 3 d of culture (original magnification×200). Red arrows indicate the cellular aggregates and black arrows indicate veil- or sheet-like processes of dendritic cells; **B**: Veil- or sheet-like processes of most cells after 7 d of culture (original magnification× 400).

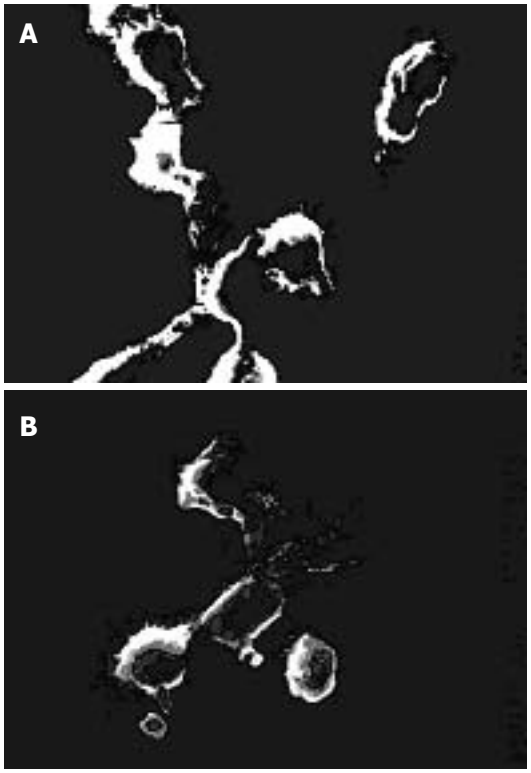

Figure 2 Cells cultivated with 10% FCS-RPMI 1640 supplemented with rhGM-CSF and rhIL-4 for 7 d (original magnification×1500).

blood was obtained from healthy adult donors.

Culture of DCs

Human peripheral blood mononuclear cells (PBMC) were isolated from buffer coat from normal donors by Ficoll-Hypaque density gradient centrifugation according to standard procedures. Monocytes were re-suspended in RPMI-10% FCS at 1×10^6 cells/mL, and allowed to adhere to 6-well plates (Costar Corp., Cambridge, MA). After a 2h adherence step at 37 °C in complete medium, nonadherent cells were washed with PBS and the remaining adherent cells were immediately subjected to the DC differentiation protocol. Briefly, monocytes were re-suspended and cultured in RPMI 1640 supplemented with 10% FCS, 25 mmol/L HEPES, and 2 mmol/L glutamine (complete medium) containing 1000 U/mL rhGM-CSF and 1000 U/mL rhIL-4. Cells were cultured for seven days, with cytokine addition every two days to obtain DCs (before incubation, a piece of 12mm × 12mm sterile coverslip was put into one of the plates and used for scanning electronic microscopy)

Morphological observation of cultured cells

Cells were observed under light microscope during incubation. After seven-day culture, the coverslip was covered with DCs and prefixed with a 2% glutaraldehyde solution for 1 h at 4 °C. Post-fixation was done with a 2% osmium tetroxide solution for 30 min at 4 °C. After each fixation, the samples were washed twice in PBS containing BSA, dehydrated in graded ethanol and dried at "critical point" in liquid CO_2 under 95-bar pressure. Then, the coverslip was covered with gold by cathodic spraying. Finally, the samples were examined under scanning electron microscope (S-520, HITACHI).

Immunofluorescence staining of DC-SIGN

Cells to be used for staining with antibody were Fc-blocked cells by treatment with 1μg of BSA/10^5 cells for 15 min at room temperature, without washing the excess blocking BSA from this reaction. Then 25 μL of the Fc-blocked cells (1×10^5 cells) was transferred to a 5 mL tube, and 10 μL of PE-conjugated anti-DC-SIGN reagent was added. After incubated for 30-45 min at 4 °C in the dark, un-reacted anti-DC-SIGN reagent was removed by washing the cells twice in 4 mL of isotonic phosphate buffer supplemented with 0.5% BSA by centrifugation at 500 r/min for 5 min. The cell pellet was re-suspended in 100 μL of PBS buffer and allowed to adhere to poly-L-lysine-coated coverslips for 30 min at 4 °C in darkness for final immunofluorescence. The representative fields of cells were photographed through an oil immersion lens on a Leica DM 6000B microscope (Leica Microsystems, Wetzlar, Germany).

RESULTS

Morphology of cultivated DCs

Phase-contrast observation: Three cellular aggregates were attached to a layer of adherent cells (Figure 1A). Some of the profiles in the aggregates had veil- or sheet-like processes of DCs. On d 7 of culture, these adherent

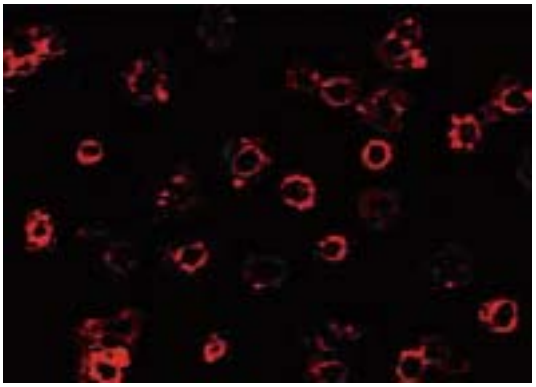

Figure 3 Cells stained with monoclonal anti-human DC-SIGN phycoerythrin (original magnification×400).

cells emigrated from the clusters, came off the surface and many typical DCs were seen floating in the culture medium (Figure 1B).

Scanning electron microscopy: Further morphological observations were carried out under scanning electron microscope (SEM). These cells had a variety of cell shapes such as bipolar elongated cells, elaborate stellate cells and DCs. Most pseudopods were long and uniform in width with blunt terminations, but smaller spinous processes were also evident (Figure 2). This phenomenon indicated that DCs had a variety of branching forms, and constantly extended and retracted many fine cell processes. The adjective "dendritic" might be appropriate for this particular cell type(Figure 3).

Determination of DC-SIGN expression

The DC-SIGN expression was detected by immunofluorescence staining with monoclonal anti-human DC-SIGN phycoerythrin. Most of the cultured cell surfaces were stained red, exhibiting a high level of expression of DC-SIGN on the surface of DCs.

DISCUSSION

In this study, we generated DCs from human PBMC with 10% FCS-RPMI 1640 supplemented with rhGM-CSF and rhIL-4. DC-SIGN expression was found on the surface of DCs.

DCs are specialized and co-stimulatory cells which play an important role in the induction of cellular immune response to antigens. DCs interact with pathogens using conserved pattern-recognition receptors, which recognize characteristic molecular arrangements within microbial carbohydrates, lipids and nucleic acids[5]. Receptors of this type include Toll-like receptors (TLRs)[6,7] and C-type lectins[8]. TLRs can transfer information about the interacting pathogens into DCs through intracellular signaling cascades, thereby eliciting appropriate cellular processes, such as DC maturation and induction of anti-inflammatory cytokines[5,6,9]. By contrast, C-type lectins recognize pathogens through their carbohydrate structure and internalize the pathogens for antigen processing and presentation[10,11]. Pathogens survive and evade the anti-microbial defense system of the host by subverting the function of these pattern-recognition receptors. Hence, an understanding of the interaction of pathogens with these receptors is essential for the design of approaches to combat infections.

DC-SIGN is a type II membrane protein with a C-type lectin extracellular domain[12]. DC-SIGN plays an important role in establishing the initial contact between DCs and resting T lymphocytes through its recognition of ICAM-3, and mediates DC trafficking through interactions with endothelial ICAM-2[13]. Therefore, DC-SIGN appears to be a critical mediator of the migratory cell and T cell-interacting capabilities. It has been identified that DC-SIGN helps HIV-1 to subvert DC functions and infect the host.

Recently, it was found that DC-SIGN is also an HCV envelope binding receptor and the HCV envelope glycoprotein E2 can bind to DC-SIGN through high-mannose N-glycans[14]. It is becoming increasingly clear that other pathogens including cytomegalovirus (CMV), Ebola, SARS coronavirus, *Helicobacter pylori*, mycobacterium tuberculosis, *Leishmania*, *schistosoma mansoni*, *etc,* can subvert DC functions to escape immune surveillance[15]. Therefore, investigation of the mechanisms of DC-mediated T lymphocyte priming, tolerance, effector cell migration and function may be helpful in restricting HCV dissemination and infection.

REFERENCES

1 **Cambi A**, Figdor CG. Dual function of C-type lectin-like receptors in the immune system. *Curr Opin Cell Biol* 2003; **15**: 539-546

2 **Gurney KB**, Elliott J, Nassanian H, Song C, Soilleux E, McGowan I, Anton PA, Lee B. Binding and transfer of human immunodeficiency virus by DC-SIGN+ cells in human rectal mucosa. *J Virol* 2005; **79**: 5762-5773

3 **Lozach PY**, Amara A, Bartosch B, Virelizier JL, Arenzana-Seisdedos F, Cosset FL, Altmeyer R. C-type lectins L-SIGN and DC-SIGN capture and transmit infectious hepatitis C virus pseudotype particles. *J Biol Chem* 2004; **279**: 32035-32045

4 **Lozach PY**, Burleigh L, Staropoli I, Navarro-Sanchez E, Harriague J, Virelizier JL, Rey FA, Desprès P, Arenzana-Seisdedos F, Amara A. Dendritic cell-specific intercellular adhesion molecule 3-grabbing non-integrin (DC-SIGN)-mediated enhancement of dengue virus infection is independent of DC-SIGN internalization signals. *J Biol Chem* 2005; **280**: 23698-23708

5 **Janeway CA**, Medzhitov R. Innate immune recognition. *Annu Rev Immunol* 2002; **20**: 197-216

6 **Underhill DM**, Ozinsky A. Phagocytosis of microbes: complexity in action. *Annu Rev Immunol* 2002; **20**: 825-852

7 **Thoma-Uszynski S**, Stenger S, Takeuchi O, Ochoa MT, Engele M, Sieling PA, Barnes PF, Rollinghoff M, Bolcskei PL, Wagner M, Akira S, Norgard MV, Belisle JT, Godowski PJ, Bloom BR, Modlin RL. Induction of direct antimicrobial activity through mammalian toll-like receptors. *Science* 2001; **291**: 1544-1547

8 **Weis WI**, Taylor ME, Drickamer K. The C-type lectin superfamily in the immune system. *Immunol Rev* 1998; **163**: 19-34

9 **Wang QC**, Feng ZH, Zhou YX, Nie QH. Induction of hepatitis C virus-specific cytotoxic T and B cell responses by dendritic cells expressing a modified antigen targeting receptor. *World J Gastroenterol* 2005; **11**: 557-560

10 **Figdor CG**, van Kooyk Y, Adema GJ. C-type lectin receptors on dendritic cells and Langerhans cells. *Nat Rev Immunol* 2002; **2**: 77-84

11 **Feng ZH**, Wang QC, Nie QH, Jia ZS, Zhou YX. DC-SIGN: binding receptor for HCV? *World J Gastroenterol* 2004; **10**: 925-929

12 **Engering A**, Geijtenbeek TB, van Vliet SJ, Wijers M, van Liempt E, Demaurex N, Lanzavecchia A, Fransen J, Figdor CG, Piguet V, van Kooyk Y. The dendritic cell-specific adhesion receptor DC-SIGN internalizes antigen for presentation to T cells. *J Immunol* 2002; **168**: 2118-2126
13 **Geijtenbeek TB**, Krooshoop DJ, Bleijs DA, van Vliet SJ, van Duijnhoven GC, Grabovsky V, Alon R, Figdor CG, van Kooyk Y. DC-SIGN-ICAM-2 interaction mediates dendritic cell trafficking. *Nat Immunol* 2000; **1**: 353-357
14 **Lozach PY**, Lortat-Jacob H, de Lacroix de Lavalette A, Staropoli I, Foung S, Amara A, Houles C, Fieschi F, Schwartz O, Virelizier JL, Arenzana-Seisdedos F, Altmeyer R. DC-SIGN and L-SIGN are high affinity binding receptors for hepatitis C virus glycoprotein E2. *J Biol Chem* 2003; **278**: 20358-20366
15 **Li Jun**, Feng Zhi-Hua. Update of DC-SIGN and Pathogen Infection Study. *Shijie Huaren Xiaohua Zazhi* 2005; **13**: 662-666

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RAPID COMMUNICATION

Relationship between co-stimulatory molecule B7-H3 expression and gastric carcinoma histology and prognosis

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Abstract

AIM: To investigate the expression of co-stimulatory molecule B7-H3 in gastric carcinoma and adenoma tissue as well as normal gastric tissue and to explore the relationship between B7-H3 expression and pathological features and prognosis of gastric carcinoma.

METHODS: B7-H3 expression was detected in 102 samples of human gastric carcinoma and 10 samples of gastric adenoma and 10 samples of normal gastric tissue by immunohistochemical assay. Correlation between the expression of B7-H3 and the patients'age, sex, gastric carcinoma locus, tumor size, tissue type, tumor infiltration depth, differentiation degree, lymph node metastasis, and survival time was analyzed.

RESULTS: B7-H3 was expressed in all gastric adenoma samples and in 58.8% samples of gastric carcinoma. B7-H3 expression in gastric carcinoma samples was not related with the patients' age, sex, lymph node metastasis, and tumor size ($P > 0.05$), but with the survival time, infiltration depth of tumor and tissue type.

CONCLUSION: Detection of B7-H3 expression in gastric carcinoma tissue is beneficial to the judgment of the prognosis of gastric carcinoma patients and the choice of treatment.

Key words: B7-H3 expression; Gastric carcinoma; Gastric adenoma; Prognosis

Wu CP, Jiang JT, Tan M, Zhu YB, Ji M, Xu KF, Zhao JM, Zhang GB, Zhang XG. Relationship between co-stimulatory molecule B7-H3 expression and gastric carcinoma histology and prognosis. *World J Gastroenterol* 2006; 12(3): 457-459

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INTRODUCTION

Tumor immune response is a very complicated physiological process involving many immune cells and molecules including membranous molecules and dissolubility factors. Studies indicate that a group of cell membrane molecules (co-stimulatory molecules) play a very important role in tumor immune response in the place of adjusted expression, interaction, and signal transmission. Co-stimulatory molecules are divided into two groups: TNF-TNF receptor superfamily and immunoglobulin superfamily. B7 family can transmit signals to co-stimulatory molecules of T cells[1]. B7RP-1 (B7H, B7-H2), B7-H1 (PD-L1), B7-DC (PD-L2) and B7-H3 have been found and make it more complex to adjust the effects of this family[2,3]. B7-H3 is a new co-stimulatory member of the B7 family and shares 20-27% identical amino acids with other members of the B7 family[4]. It was reported that B7-H3 could stimulate CD4+ and CD8+T cells to increase the activity of CTL[5]. B7-H3 is regarded as a positive regulation molecule. To our knowledge, expression of B7-H3 in gastric carcinoma has not been previously demonstrated.

MATERIALS AND METHODS

Materials

From 1998 to 1999, 102 samples were collected from gastric cancer patients who had undergone surgery in our hospital. The tumor was located in the mucosa in 10 cases, in shallow muscularis in 20 cases and in deep muscularis in 72 cases. The tumors were classified into well-, moderately- and poorly-differentiated adenocarcinomas. None of the patients received chemotherapy or radiotherapy before the surgery. We also investigated 10 specimens from gastric adenoma patients who had undergone surgery between 1998 and 1999.

The study was approved by the ethics committee of our hospital and all patients gave their written informed consent prior to enrolment.

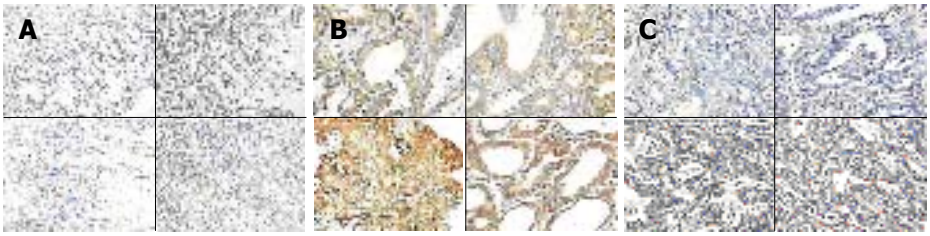

Figure 1 Staining of B7-H3 in normal gastric tissue (**A**), gastric adenoma tissue (**B**), and gastric carcinoma tissue (**C**).

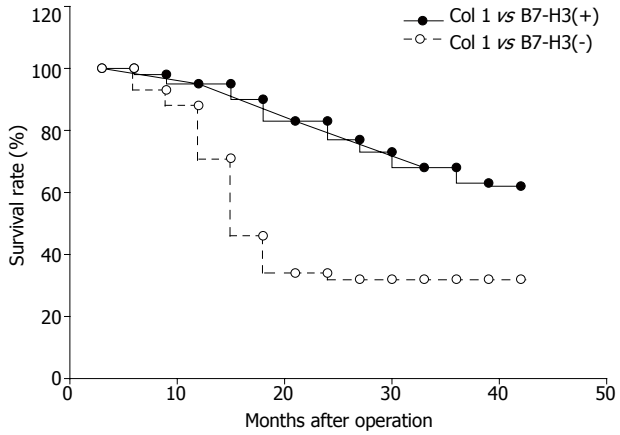

Figure 2 Correlation between B7-H3 expression and death of gastric carcinoma patients.

Immunohistochemical staining and assessment

Labeling was carried out with Elivision™ plus kit. The samples were fixed in formalin, embedded with paraffin wax and cut into 3-μm-thick sections. The sections were dewaxed and rinsed and washed thrice with PBS (pH 7.4) for 3 min. The antigen of tissue was repaired and one drop or 50 μL of 3% hydrogen peroxidase solution was added to each section and incubated at room temperature for 10 min to eliminate endogenous peroxidase activity. The sections were washed thrice with PBS for 3 min again. PBS was discarded; one drop or 50 μL of primary antibody was added to each section and incubated at room temperature for 10 min or placed overnight at 40 ℃. The sections were washed thrice with PBS for 5 min. PBS was discarded, one drop or 50 μL of polymer accentuator (reagent A) was added to each section and incubated at room temperature for 20 min. After being washed thrice with PBS for 3 min each time, PBS was discarded and one drop or 50 μL of enzyme-labeled anti-mouse/rabbit polymer (reagent B) was added to each section and incubated at room temperature for 30 min. The sections were washed thrice with PBS for 3 min each time. PBS was discarded; two drops or 100 μL of freshly prepared DBA coloration fluid was added to each section and examined under microscope for 3-10 min. All the samples were fixed in formalin, embedded with paraffin wax and cut into 3-μm-thick sections. The samples were assessed blindly by calculating the average ratio of positive cells in 10 vision fields (the plasma was stained brown-yellow) under a 400× microscope. If the average positive cell ratio was more than 20%, the sample was considered positive.

Statistical analysis

Difference between the groups was evaluated by χ^2 test using SPSS version 10.0 for Windows and the relationship between the prognosis and various factors was evaluated by multivariable logistic regression. All P values were based on two-sided testing. $P<0.05$ was considered statistically significant.

RESULTS

B7-H3 expression in gastric cancer and adenoma

B7-H3 was positively expressed in cell membrane and cytoplasm of gastric cancer and adenoma cells (Figure 1). B7-H3 was expressed in all the 10 specimens of gastric adenoma tissue and in 58.8% samples of gastric cancer tissue.

Relationship between B7-H3 expression and age, sex, and prognosis of patients

B7-H3 expression in gastric carcinoma tissue was not related with the patients'age and sex ($P>0.05$, Table 1). The association of B7-H3 expression with the survival time after surgery indicated that B7-H3 expression was related to the prognosis of patients (Figure 2). The positive rate (74.5%) of B7-H3 expression was higher in gastric cancer patients who survived more than 5 years than in those who survived less than 2 years (43.1%, $P<0.01$).

Relationship between B7-H3 expression and pathological features of gastric cancer

B7-H3 expression was not related with lymph node metastasis, tumor location and size ($P>0.05$), but with survival time, infiltration depth of tumor and histology type (Table 1).

Related factors of gastric cancer patients' survival time

Univariate analysis suggested that the infiltration depth of tumor, survival time of patients and histology type of gastric cancer were related to B7-H3 expression. After adjustment for these factors, gastric cancer patients with high intratumor B7-H3 expression could survive 2-fold longer than those with low intratumor B7-H3 expression (risk ratio = 2.803; 95%CI = 1.051-7.477; $P=0.040$), suggesting that B7-H3 expression might be an independent factor affecting the survival time of gastric cancer patients.

DISCUSSION

Human B7-H3 is also known as B7 relative protein 2 (B7RP-2) and its gene map on chromosome 15 is composed of seven exons and six introns. Mature B7-H3 protein has 316 amino acids and its molecular weight is 45-66 ku. It belongs to immunoglobulin superfamily and is a type Ⅰ transmembrane protein composed of

Table 1 Correlation between tumor B7-H3 expression and pathologic features of gastric carcinoma patients

Features	Total cases (*n*)	Positive cases (*n*)	Positive rate (%)
Tumor location			
Top 1/3 layer of stomach	31	19	61.2
Middle 1/3 layer of stomach	30	17	56.7
Bottom 1/3 layer of stomach	41	24	58.5
Degree of differentiation			
Well-differentiated	72	48	66.7
Poorly-differentiated	30	12	40.0
Infiltration depth without			
Infiltration at deep muscular layer	30	23	76.7
With infiltration at deep muscular layer	72	37	51.3
Lymph node metastasis			
Negative	54	33	61.1
Positive	48	27	56.3
Survival time			
<2 years	51	22	43.1
>5 years	51	38	74.5
Primary tumor size			
<5 cm	55	35	63.6
>5 cm	47	25	53.2

extracellular, transmembrane and intracellular regions. B7-H3 has been recently identified as a new co-stimulatory member of the B7 family and shares 20-27% identical amino acids with other members of the B7 family[4]. It is extensively expressed in non-lymphoid tissues including the heart, liver, prostate, placenta, testis, pancreas, small, and large intestine and also in some tumor cell lines such as G361, HeLa S3, K562, A546, and SW480. B7-H3 was expressed in all the 10 specimens of gastric adenoma tissue and in 58.8% samples of gastric cancer tissue. We also found that B7-H3 was highly expressed in some epithelial tumor cell lines such as M435, A549, and H01299. A recent study found that B7-H3 can stimulate CD4+ and CD8+T cells to increase the activity of CTLs[5]. In addition, B7-H3 increases the secretion of IFN-γ and can upregulate IL-8 and TNF-α. When B7-H3Ig is blocked by anti-B7-H3 multi-clone antibody, secretion of IFN-γ by DCs can be downregulated, indicating that B7-H3 is a positive regulatory molecule.

Our study suggested that B7-H3 expression in gastric carcinoma tissue was not related with the age and sex of patients, lymph node metastasis and size of tumor but with the survival time of patients, infiltration depth and histology type of tumor. The positive rate (74.5%) of B7-H3 expression was higher in gastric cancer patients who survived more than 5 years than in those who survived less than 2 years (43.1%), suggesting that B7-H3 expression is an independent factor affecting the survival time of gastric cancer patients and that B7-H3 might act as a positive regulatory factor in tumor immunology.

Sun *et al.*[6] showed that intratumor injection of a mouse B7-H3 pcDNA3 expression plasmid leads to complete regression of 50% tumors and can significantly inhibit tumor growth. Mice with their tumors completely regressed can resist a challenge with parental tumor cells. B7-H3-mediated anti-tumor immunity is mediated by CD8(+) T and NK cells, rather than CD4(+) T cells. These results indicate that B7-H3 interactions play a role in regulating cell-mediated immune responses against cancer.

However, Suh *et al.*[7] found that B7-H3 inhibits immune response by inhibiting type 1 [T(H)1] responses and production of IFN-γ. A recent study showed that B7-H3 induces T cell proliferation and IFN-γ production through non co-stimulatory pathways[8], indicating that B7-H3 may have more than one receptor on activated T cells.

Because the known receptor of B7-H3 has not been found, its function in the immune response is not clear. B7-H3 is extensively expressed in peripheral tissues, suggesting that it may play an important role in inflammation and transplantation immune response. B7-H3 is highly expressed in epithelial tumor cell lines and positively related to the prognosis of gastric cancer patients, indicating that B7-H3-deficient expression in tumor tissues may be closely associated with tumor immune escape.

In conclusion, B7-H3 is related to the development of gastric cancer and acts as an independent index for the diagnosis and prognosis of gastric cancer. Further study is needed to explore the exact relationship between B7-H3 and gastric cancer.

REFERENCES

1 **Pardoll DM**. Spinning molecular immunology into successful immunotherapy. *Nat Rev Immunol* 2002; **2**: 227-238
2 **Dong H**, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat Med* 1999; **5**: 1365-1369
3 **Tseng SY**, Otsuji M, Gorski K, Huang X, Slansky JE, Pai SI, Shalabi A, Shin T, Pardoll DM, Tsuchiya H. B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. *J Exp Med* 2001; **193**: 839-846
4 **Ayrapetov MK**, Lee S, Sun G. Expression, purification, and biochemical characterization of Chk, a soluble protein tyrosine kinase. *Protein Expr Purif* 2003; **29**: 148-155
5 **Ling V**, Wu PW, Spaulding V, Kieleczawa J, Luxenberg D, Carreno BM, Collins M. Duplication of primate and rodent B7-H3 immunoglobulin V- and C-like domains: divergent history of functional redundancy and exon loss. *Genomics* 2003; **82**: 365-377
6 **Sun X**, Vale M, Leung E, Kanwar JR, Gupta R, Krissansen GW. Mouse B7-H3 induces antitumor immunity. *Gene Ther* 2003; **10**: 1728-1734
7 **Suh WK**, Gajewska BU, Okada H, Gronski MA, Bertram EM, Dawicki W, Duncan GS, Bukczynski J, Plyte S, Elia A, Wakeham A, Itie A, Chung S, Da Costa J, Arya S, Horan T, Campbell P, Gaida K, Ohashi PS, Watts TH, Yoshinaga SK, Bray MR, Jordana M, Mak TW. The B7 family member B7-H3 preferentially down-regulates T helper type 1-mediated immune responses. *Nat Immunol* 2003; **4**: 899-906
8 **Steinberger P**, Majdic O, Derdak SV, Pfistershammer K, Kirchberger S, Klauser C, Zlabinger G, Pickl WF, Stöckl J, Knapp W. Molecular characterization of human 4Ig-B7-H3, a member of the B7 family with four Ig-like domains. *J Immunol* 2004; **172**: 2352-2359

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RAPID COMMUNICATION

Clinical characteristics and prognostic factors of splenic abscess: A review of 67 cases in a single medical center of Taiwan

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Abstract

AIM: To analyze 67 cases of splenic abscess in a medical center of Taiwan during a period of 19 years.

METHODS: From January 1986 to December 2004, a total of 67 patients with splenic abscess were enrolled for the retrospective study. The clinical characteristics, underlying diseases, organism spectra, therapeutic methods, APACHE II scores, and mortality rates were analyzed.

RESULTS: There were 41 males and 26 females with the mean age of 54.1 ± 14.1 years. Multiple splenic abscesses (MSA) account for 28.4% and solitary splenic abscess in 71.6% of the patients. Twenty-six of sixty-seven patients (35.8%) had extrasplenic abscesses, with leading site of liver (34.6%). Microbiological cultures were positive in 58 patients (86.6%), with 71.8% in blood culture and 93.5% in abscess culture. Gram negative bacillus (GNB) infection predominated (55.2%), with leading pathogen of *Klebsiella pneumoniae* (22.4%), followed by gram positive coccus (GPC) infection (31%). Splenectomy was performed in 26 patients (38.8%), percutaneous drainage or aspiration in 21 (31.3%), and antibiotic therapy alone in 20 patients (29.9%). Eventually, 12 of 67 patients expired (17.9 %). By statistics, spleen infected with GNB was likely to develop multiple abscesses compared with infection with GPC ($P = 0.036$). Patients with GNB infection ($P = 0.009$) and multiple abscesses ($P = 0.011$) experienced a higher mortality rate than patients with GPC infection and solitary abscess. The mean APACHE II score of 12 expired patients (16.3 ± 3.2) was significantly higher than that of the 55 survivals (7.2 ± 3.8) ($P < 0.001$).

CONCLUSION: MSA, GNB infection, and high APACHE II scores are poor prognostic factors. Early surgical intervention should be encouraged when these risk factors are present.

Key words: Splenic abscess; Prognosis; Gram negative bacillus infection; APACHE II scores

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INTRODUCTION

Splenic abscess is a rare entity, with a reported frequency in autopsy series between 0.14% and 0.7%[1,2,3]. It remains a subject of case reports and of small institutional series. Large case numbers reviewed in a single institute was rare and difficult. However, reviews from different series of geographic localizations and populations might be obscure on pathologic features in local area. Further, splenic abscess often occurs in the patients with underlying diseases[4-8]. Patients with multiple splenic abscesses (MSA) or immunodeficiency are considered to have a poor prognosis and high mortality[4,5,9]. Many surgeons had reported that splenectomy is a better way for treatment of splenic abscess[3-5,8-10]. Recently, medical treatment and abscess drainage are proved to be efficient methods in the treatment of splenic abscess[11-14]. However, various conditions interfere with the prognosis of splenic abscess, such as underlying diseases, abscess number and size, organism spectra, therapeutic methods and general conditions. There is no single risk factor that can predict

Table 1 Comparison of epidemiology and symptomatology in the reviews

Variable	Chun *et al*[17] 1900-1977	Nelken *et al*[5] 1977-1986	Ooi *et al*[18] 1987-1995	Present study 1986-2004
Number of cases	173	189	287	67
Male:female ratio(%)	104:61 (63)	125:64 (66)	163:80 (67)	41:26 (61)
Mean age (yr)	36.8	Not available	41.1	54.1
Age range (yr)	6 mo-83	6 mo-82	6 mo-92	19-79
Clinical presentations				
Fever (%)	95.4	84	90.8	85
Left upper quadrant pain(%)	42.1	39	49.8	43.3
Splenomegaly(%)	53.9	40	30.7	67.2
Left pleural effusion(%)	19.7	Not available	22.3	41.5

the prognosis exactly till now. APACHE II score is a method to evaluate the general condition and to easily get the score from the patient's general data[15]. It has not been adopted in the evaluation of splenic abscess. To further elucidate the prognostic factors in splenic abscess, we have analyzed the various risk factors (including APACHE II score) of splenic abscess in a large series of 67 cases from a single medical center during a period of 19 years.

MATERIALS AND METHODS

From January 1985 to December 2004, a total of 67 patients with splenic abscess were enrolled. The diagnosis of splenic abscess was made if one of the following criteria was met: (1) microbiologically documented abscess (blood or splenic aspirate) with compatible splenic imaging studies of computed tomography (CT) or ultrasonography (US); (2) Pathologic microscopic examination of the spleen on autopsy, resection or aspirate that revealed abscess formation; (3) Operative findings of splenic abscess on exploratory laparotomy; (4) In the presence of typical clinical manifestations and finding of CT or US, the patients' conditions regressed after antibiotic therapy in cases without interventional therapies (only three cases in this study). Patients with diseases progressing to death and sterile culture were excluded from this study. The demographic, clinical, and laboratory characteristics, underlying diseases, organism spectra, abscess number and size, therapeutic methods, patients' APACHE II scores, and mortality rates were collected and analyzed. The size of abscess is recorded as the largest diameter in either solitary or multiple abscesses. The APACHE II score was evaluated following the criteria of Knaus' report[15]. Age, chronic health problem score, Glasgow coma score, and 12 physiologic variables (vital signs, oxygenation, laboratory values; the most severely abnormal of each in the first 24 h of admission) were used to make a sum of scores[15]. All the survival cases had been followed clinically for more than 6 mo. Comparison between groups of independent samples was assessed by the Student's *t*-test, one-way ANOVA (*post hoc* multiple comparisons by Scheffe's procedure). The associations between categorical variables were assessed using the χ^2 or Fisher's exact test. A two-tailed $P<0.05$ was considered statistically significant.

RESULTS

There were 41 males and 26 females with the mean age of 54.1±14.1 years (range 19-79 years). The mean age of males was 51.9±13.6 years, and that of the females was 57.4±14.5 years ($P=0.123$). Sizes of abscess ranged from 1.5 to 16 cm, with a mean of 4.02±2.56 cm.

The symptoms were fever in 57 patients (85.1%), left upper quadrant pain in 29 patients (43.3%), diffuse abdominal pain in 10 patients (14.9%), left chest wall pain in 3 patients (9%), and dyspnea in 5 patients (7.5%). Twenty five patients (37.3%) suffered from more than two symptoms at the time of diagnosis. The physical examination revealed splenomegaly in 34 patients (50.7%), left upper quadrant tenderness in 30 patients (44.7%), generalized abdominal tenderness in 9 patients (13.4%), left chest basilar rales in 6 patients (8.9%), and left chest basilar dullness in 5 patients (7.5%), leukocytosis over 10 000/mm^3 in 47 patients (70.1%), and leucopenia in 3 patients (4.5%) (Table 1).

Among the 67 patients, 54 of them (80.6%) had predisposing underlying diseases. Diabetes mellitus (DM) was the leading disease (25 patients, 46.3%). Seven of 54 patients (12.9%) had more than two underlying diseases simultaneously (Table 2). Statistical analysis revealed that the presence of underlying disease did not correlate with any clinicopathologic parameter, including size of abscess, numbers of abscess, age, gender, species of microorganism, and mortality rates of patients.

Of the images of splenic abscess, 65 patients underwent chest roentgenogram study. Forty of sixty-five patients (61.5%) had abnormalities as left lower lung infiltration (13 cases, 20%) or left pleural effusion (27 cases, 41.5%). Further, 66 of 67 patients underwent abdominal US and/or abdominal CT studies, in which 46 patients received both US and CT, 9 patients received US alone, and 11 patients received CT alone. One who had a solitary splenic abscess (SSA) had no image study because of emergent operation for peritonitis. The sensitivity of US and CT was 98.18% and 98.24%, respectively. Splenomegaly was found by images in 45 patients (67.2%). There were MSA in 19 cases (28.4%) and SSA in 48 cases (71.6%) (Table 3). Gas formation in the abscess was found in only 8 patients (11.9%), mainly in patients with gram negative bacillus (GNB) infections (7 cases). In addition

Table 2 Underlying and predisposing diseases in 67 cases of splenic abscess

Underlying diseases (n = 67)	Solitary (SSA) (n = 48)	Multiple (MSA) (n = 19)	Extrasplenic abscess (n = 26)
DM (20)	17	3	10[1]
Endocarditis (7)	7	0	4[2]
Pancreatitis (5)	2	3	2[3]
Pancreatitis+DM (2)	1	1	0
Pancreatic cancer (1)	1	0	0
Liver cirrhosis (2)	1	1	1[4]
Trauma (2)	1	1	0
MDS (2)	0	2	2[5]
SLE (2)	2	0	0
CHF (1)	1	0	0
Perforated peptic ulcer (1)	1	0	0
Colon perforation+ DM (1)	1	0	1[6]
Endocarditis+DM (1)	1	0	0
Biliary tract stone	1	0	0
AIDS (1)	0	1	1[7]
ALL (1)	0	1	0
Aplastic anemia (1)	1	0	0
ESRD+ovarian cancer (1)	0	1	0
COPD+CHF (1)	1	0	0
Lung cancer+DM (1)	1	0	0
Unknown (13)	8	5	5[8]

DM, diabetes mellitus; MDS, myelodysplastic syndrome; SLE, systemic lupus erythematosus; CHF, congestive heart failure; AIDS, acquired immunodeficiency syndrome; ALL, acute lymphocytic leukemia; ESRD, end stage renal disease; COPD, chronic obstructive pulmonary disease. [1]Liver: 3; subphrenic area: 2; pancreas: 1; anus: 1; gluteal muscle: 1; retroperitoneal cavity: 1; brain+lung: 1. [2]brain: 2; aortic valve: 1; lung: 1. [3]pancreas: 2. [4]gluteal muscle: 1. [5]liver: 2. [6]subphrenic area: 1. [7]liver: 1. [8]liver: 2; brain+lung: 1; retroperitoneal cavity: 1; subphrenic area: 1.

Table 3 Microorganisms of blood culture and abscess culture of splenic abscess

Microorganism (n = 67)	Solitary (n = 48)	Multiple (n = 19)	Extrasplenic abscess (n = 26)
Aerobes			
Gram positive-18			
Streptococcus viridans (SV) (8)	7	1	2[1]
Staphylococcus aureus (5)	5	0	3[2]
Enterococcus species (5)	5	0	1[3]
Gram negative-32			
Escherichia coli (11)	8	3	4[4]
Klebsiella pneumoniae (KP)(10)	4	6	7[5]
Pseudomonas species (PS) (5)	4	1	1[6]
Salmonella species (5)	4	1	2[7]
Proteus (1)	1	0	0
Gram positive+gram negative-4			
SV+KP (2)	2	0	1[8]
SV+PS (1)	0	1	1[9]
SV+PS+KP (1)	0	1	0
Anaerobes-2			
Gram positive			
Propionibacterium acnes (1)	1	0	0
Gram negative			
Bacteroides fragilis (1)	1	0	1[10]
Mycobacterium-1			
Tuberculosis (1)	0	1	1[11]
Fungus-1			
Cryptococcus neoformans (1)	0	1	1[12]
Sterile culture-9	6	3	1[13]

[1]Brain: 1; liver: 1. [2]lung: 1; retroperitoneum: 1; brain: 1. [3]retroperitoneum: 1. [4]liver: 1; subphrenic area: 1; gluteal muscle: 1; pancreas: 1. [5]liver: 4; brain+lung: 2; subphrenic area: 1. [6]anus: 1. [7]aortic valve: 1; subphrenic area: 1. [8]subphrenic area: 1. [9]gluteal muscle (1). [10]pancreas: 1. [11]liver: 1. [12]liver: 1. [13]pancreas: 1.

to spleen, 26 of 67 patients (35.8%) had extrasplenic abscesses, with leading site of liver (Table 4). Among them two patients showed two sites of extrasplenic abscesses in the lung and brain. By statistics, we found that MSA tended to develop in non-diabetic patients ($P = 0.053$), and in abscess with GNB infection ($P = 0.036$, Tables 2 and 3). Furthermore, MSA patients experienced a higher mortality rate than SSA patients ($P = 0.011$, Table 5). Seven of nineteen (36.8%) MSA patients, in contrast to 5 of 48 (10.4%) SSA patients expired eventually. *Klebsiella pneumoniae* (*K. pneumoniae*) (70%) and staphylococcus aureus (60%) were most common pathogens to develop extrasplenic abscess (Table 3). However, statistical analysis revealed no significant correlation between the presence of extrasplenic abscess and any clinicopathologic factor.

Bacteriologic studies revealed that positive microbiological cultures were found in 58 patients (86.6%), and sterile in the other 9 patients (Table 3). Blood culture was positive in 46 of 64 patients (71.8%), and abscess culture in 43 of 46 patients (93.5%). Twenty-eight of thirty patients who had both positive blood and abscess cultures revealed identical pathogens. The microorganisms of 58 cases of positive culture were predominantly GNB (32 cases, 55.2%), with *K. pneumoniae* as the leading pathogen (22.4%, Table 3). Statistical analysis revealed that abscesses with GNB were likely to develop multiple focus compared with abscesses with gram positive coccus (GPC) ($P = 0.036$). There were 11 MSA in 32 patients (34.4%) with GNB. In contrast, only one of the 18 patients (5.6%) with GPC had MSA. Furthermore, patients with GNB infection also experienced a higher mortality rate than patients with GPC ($P = 0.009$). Ten of 32 GNB (31.2%) patients, in contrast to none of 18 (0%) GPC patients expired eventually (Table 5).

In the treatment of splenic abscess, all patients received antibiotic therapy. Splenectomy was performed in 26 patients (38.8%), percutaneous drainage or aspiration in 21 (31.3%), and antibiotic therapy alone in 20 patients (29.9%). Eventually, 12 patients expired (17.9%) at the end of follow-up. Three patients with percutaneous drainage or aspiration initially were finally referred for splenectomy, and all of them survived. Two mortality patients who underwent splenectomy died of predisposing factors as extensive pancreatitis and severe abdominal trauma rather than splenic abscess itself. The mortality rate was 7.7% in patients treated with splenectomy, 28.6% in patients with percutaneous drainage or aspiration, and 20% in patients with antibiotic therapy alone. There was no difference of

Table 4 Locations of extrasplenic abscesses

Location	Case number (%)
Liver	9 (34.6)
Subphrenic area	4 (15.4)
Pancreas	3 (11.5)
Brain	3 (11.5)[1]
Lung	3 (11.5)[1]
Retroperitoneal cavity	2 (7.7)
Gluteal muscle	2 (7.7)
Aortic valve	1 (3.85)
Anus	1 (3.85)

[1]Twenty-six patients had single, and two patients had two sites of extrasplenic abscesses (lung+brain).

Table 5 Prognostic factors of splenic abscess

Variables	Category (*n*)	Outcome		*P*
		Cure (*n* = 55, %)	Mortality (*n* = 12, %)	
Gender	Female (26)	21 (81)	5 (19)	NS[1]
	Male (41)	34 (83)	7 (17)	
Abscess number*	Solitary (48)	43 (89)	5 (11)	0.011[1]
	Multiple (19)	12 (63)	7 (37)	
Extrasplenic abscesses	Without (43)	36 (84)	7 (16)	NS[1]
	With (24)	19 (79)	5 (21)	
Underlying diseases	Without (15)	12 (80)	3 (20)	NS[1]
	With (52)	43 (83)	9 (17)	
Diabetes	Without (41)	33 (80)	8 (20)	NS[1]
	With (26)	22 (85)	4 (15)	
Microorganism[a]				
	GNB (32)	22 (69)	10 (31)	0.0091[a]
	GPC (18)	18 (100)	0 (0)	
	GNB+GPC (4)	4 (100)	0 (0)	
	Others (4)	2 (50)	2 (50)	
	Sterile (9)	9 (100)	0 (0)	
Treatment	Splenectomy (26)	24 (92)	2 (8)	NS[1]
	PD or FNA+AT (21)	15 (71)	6 (29)	
	AT alone (20)	16 (80)	4 (20)	
Age		53.3±13.4	57.3±17.1	NS[2]
Size of abscess[2]		5.1±3.0	4.0±2.6	NS[2]
Leukocytes/mm[3]		13.1±4.5	9.6±5.9	NS[2]
APACHE II score*		7.3±3.8	16.1±3.2	<0.001[2]

GNB, gram negative bacillus; GPC, gram positive cocci; PD, percutaneous drainage; FNA, fine needle aspiration; AT, antimicrobial therapy. Categorical data were compared by: 1: χ^2 test or *F*-test, 2: *t*-test. [a]*P*<0.05 GNB *vs* GPC. NS: not significant.

mortality rate between the groups (Table 5). But marginally better outcome was found in patients with splenectomy than patients with percutaneous drainage or aspiration ($P=0.06$).

For analysis of patients' outcome, we first applied the APACHE II score for further correlation study. The twelve expired patients had high APACHE II scores over 15 except one. The mean score of 12 expired patients (16.1 ± 3.2) was significantly higher than that of the 55 survivals (7.3 ± 3.8) ($P<0.001$). It was also found that higher scores presented in fatal cases of each category, regardless of underlying disease, abscess number and size, organism spectrum, and different therapeutic methods (data not shown). To summarize our results, we found that patients with multiple splenic abscesses, GNB infection had a higher mortality rate than patients with solitary splenic abscess and GPC infection. Mortality patients also had a higher mean APACHE II score than survival patients (Table 5).

DISCUSSION

Reports on splenic abscess between 1900 and 1977[16], between 1977 and 1986[4], and between 1987 and 1995[17] showed a great variety of causative pathogens with a wide range of demographic and clinical conditions. Reviews of large series of patients with splenic abscess are capable of shedding light on the pathogenesis and clinical characteristics of splenic abscess[4,16,17]. Reviews from different geographic localizations and populations might obscure some specific pathologic features in the local area. Therefore, our large series review in single medical center might provide a further insight of the pathogenesis of splenic abscess in Taiwan.

Our patients experienced an older age, and higher percentage of splenomegaly and more left lung change (infiltration or pleural effusion) than that reported by other studies[4,16,17] (Table 1). These might be attributed to extensive studies by images. US and CT may help to detect small amount of pleural effusion which is subtle in CXR. Further, US and CT are also more sensitive to demonstrate splenomegaly than physical examination did. With a reported sensitivity of 96%, CT was considered to be superior to US (sensitivity 75-90%) for detecting splenic abscess[12,17]. However, we found that the sensitivity of both CT and US in this study was equal and extremely high (98%). Therefore, US is an easy diagnostic and therapeutic tool for splenic abscess[18].

The cause of splenic infection has been most often a metastatic infection or contiguous distant infection[4,16,17]. Recently, changing lifestyles resulted in an increasing prevalence of DM, malignancies, and immunosuppression becomes advanced therapeutic methods. These conditions constitute the increasing predisposing risk for the development of splenic abscess. Underlying diseases such as DM, endocarditis, and other diseases were reported not to be a good predictor for prognosis before. However, immunodeficiency is considered to be a poor prognostic factor and caused a high mortality[7-8]. In our series, the most common predisposing factors were still metastatic or contiguous infections. But 10 patients had immunodeficiency disorders (acquired immunodeficiency disease, myelodysplastic syndrome, systemic lupus erythematosus, acute lymphocytic leukemia, malignancies after chemotherapy, and aplastic anemia), and six of ten patients suffered from MSA and expired. Based on the limited case numbers, we cannot arrive at any conclusion about the prognostic role of immunodeficiency disorder.

But actually, we have found many mortality patients with severe illness (due to immunodeficiency) during the initial patients search for this study. They were excluded from this study mostly because of progression of disease to death with uncertain microbiological information. In these patients, multiple microabscesses were hard to be differentiated from micrometastatic or infarction lesions. Therefore, the real incidence of splenic abscess and the percentages of immunodeficient risk might be underestimated.

In contrast to previous reports (*Streptococcus* and *Staphylococcus* species predominant)[3,4,7,8,10], our series revealed GNB were the leading pathogens causing splenic abscess (55.2%). Among GNB, *K. pneumoniae* was the most frequently encountered pathogen (22.4%). It has been reported to be the most common pathogen of liver abscess in Taiwan[19]. Further, metastatic splenic infections from liver abscesses caused by *K. pneumoniae* were rare[19]. Primary splenic abscess due to *K. pneumoniae* has also been rarely addressed before. In the present study, we have found that extrasplenic abscesses existed in 26 of 67 patients (38.8%), with leading site of liver in 9 (34.6%). Among the 9 liver abscesses, *K. pneumoniae* accounted for 44% of pathogens. We wonder whether it was a sequela of metastatic infection from liver to spleen or a co-infection of both. Nevertheless, these results indicated the significant role of GNB infection in splenic abscess. By statistics, we found that patients with GNB infection were prone to develop multiple splenic abscess ($P=0.036$) and had a higher mortality rate ($P=0.009$) than patients with GPC did. It is a novel finding which was never reported.

In this series, the treatment was carried out by antibiotic therapy alone, percutaneous splenic aspiration and/or drainage, or surgical methods. The mortality rate in patients receiving antibiotic therapy alone or percutaneous splenic drainage was marginally higher than that undergoing splenectomy. This result came from (1) the patient number in each different treatment group was still small, and (2) surgical treatment was not performed in some patients, if patient's condition was too ill to tolerate an operation. Based on these, the prognosis of our patients cannot be predicted by treatment methods exactly. In fact, even the previous inferences were mostly derived from comparison of patients in different series because of the rarity of disease[5,16,17]. There is a lack of a prospective cohort study to demonstrate which therapy is superior to others. To overcome this, we first adopted APACHE II score to predict the patients' outcome. The fatal patients had higher APACHE II score (16.1 ± 3.2) than the survivals (7.3 ± 3.8) ($P<0.001$). It was also found that higher scores presented in fatal cases of each category, regardless of underlying disease, abscess number and size, organism spectrum, and different therapeutic methods. Among the nine expired patients, eight had a score of over 15. Therefore, we thought that a score over 15 was a risk point for splenic abscess, the sensitivity, specificity, positive predict value, and negative predict value would be 89%, 94%, 80%, and 97%, respectively.

In conclusion, we have obtained a novel finding of GNB serving as a leading pathogen in splenic abscess and provided evidence that multiple splenic abscesses, GNB infection, and high APACHE II scores are poor prognostic factors. Aggressive and early surgical intervention of splenic abscess should be encouraged when these risk factors are present.

REFERENCES

1 **Lawhorne TW**, Zuidema GD. Splenic abscess. *Surgery* 1976; **79**: 686-689
2 **Gadacz TR**. Splenic abscess. *World J Surg* 1985; **9**: 410-415
3 **Alonso Cohen MA**, Galera MJ, Ruiz M, Puig la Calle J, Ruis X, Artigas V, Puig la Calle J. Splenic abscess. *World J Surg* 1990; **14**: 513-56; discussion 513-56
4 **Nelken N**, Ignatius J, Skinner M, Christensen N. Changing clinical spectrum of splenic abscess. A multicenter study and review of the literature. *Am J Surg* 1987; **154**: 27-34
5 **Pomerantz RA**, Eckhauser FE, Thornton JW, Strodel WE, Knol JA, Zuidema GD. Covert splenic abscess: a continuing challenge. *Am Surg* 1986; **52**: 386-390
6 **Faught WE**, Gilbertson JJ, Nelson EW. Splenic abscess: presentation, treatment options, and results. *Am J Surg* 1989; **158**: 612-614
7 **Green BT**. Splenic abscess: report of six cases and review of the literature. *Am Surg* 2001; **67**: 80-85
8 **Phillips GS**, Radosevich MD, Lipsett PA. Splenic abscess: another look at an old disease. *Arch Surg* 1997; **132**: 1331-135; discussion 1331-135
9 **Simson JN**. Solitary abscess of the spleen. *Br J Surg* 1980; **67**: 106-110
10 **Smyrniotis V**, Kehagias D, Voros D, Fotopoulos A, Lambrou A, Kostopanagiotou G, Kostopanagiotou E, Papadimitriou J. Splenic abscess. An old disease with new interest. *Dig Surg* 2000; **17**: 354-357
11 **Goerg C**, Schwerk WB, Goerg K. Splenic lesions: sonographic patterns, follow-up, differential diagnosis. *Eur J Radiol* 1991; **13**: 59-66
12 **Tikkakoski T**, Siniluoto T, Päivänsalo M, Taavitsainen M, Leppänen M, Dean K, Koivisto M, Suramo I. Splenic abscess. Imaging and intervention. *Acta Radiol* 1992; **33**: 561-565
13 **Jang TN**, Fung CP. Treatment of pyogenic splenic abscess: nonsurgical procedures. *J Formos Med Assoc* 1995; **94**: 309-312
14 **Chou YH**, Tiu CM, Chiou HJ, Hsu CC, Chiang JH, Yu C. Ultrasound-guided interventional procedures in splenic abscesses. *Eur J Radiol* 1998; **28**: 167-170
15 **Knaus WA**, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985; **13**: 818-829
16 **Chun CH**, Raff MJ, Contreras L, Varghese R, Waterman N, Daffner R, Melo JC. Splenic abscess. *Medicine (Baltimore)* 1980; **59**: 50-65
17 **Ooi LL**, Leong SS. Splenic abscesses from 1987 to 1995. *Am J Surg* 1997; **174**: 87-93
18 **Changchien CS**, Tsai TL, Hu TH, Chiou SS, Kuo CH. Sonographic patterns of splenic abscess: an analysis of 34 proven cases. *Abdom Imaging* 2002; **27**: 739-745
19 **Ko WC**, Paterson DL, Sagnimeni AJ, Hansen DS, Von Gottberg A, Mohapatra S, Casellas JM, Goossens H, Mulazimoglu L, Trenholme G, Klugman KP, McCormack JG, Yu VL. Community-acquired Klebsiella pneumoniae bacteremia: global differences in clinical patterns. *Emerg Infect Dis* 2002; **8**: 160-166

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RAPID COMMUNICATION

Telomerase activity and human telomerase reverse transcriptase expression in colorectal carcinoma

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Abstract

AIM: To study the activity of telomerase and the expression of human telomerase reverse transcriptase (hTERT) in colorectal carcinoma and its adjacent tissues, normal mucosa and adenomatoid polyp, and to evaluate their relation with carcinogenesis and progression of colorectal carcinoma.

METHODS: Telomerase activity and hTERT expression were determined in 30 samples of colorectal carcinoma and its adjacent tissues, normal mucosa and 20 samples of adenomatoid polyp by modified telomeric repeat amplification protocol (TRAP), enzyme-linked immunosorbent assay (ELISA) and immunohistochemical method.

RESULTS: Telomerase activity and hTERT expression were 83.33% (25/30) and 76.67% (23/30) respectively in colorectal carcinoma, which were obviously higher than those in paracancerous tissues (13.33%, 16.67%), normal mucosa (3.33%, 3.33%) and adenomatoid polyp (10%, 10%). There was a significant difference between colorectal carcinoma and other tissues (P=0.027). The telomerase activity and hTERT expression were higher in colorectal carcinoma with lymphatic metastasis than in that without lymphatic metastasis (P=0.034). When the histological classification and clinical stage were greater, the telomerase activity and hTERT expression increased, but there was no significant difference between them. In colorectal carcinoma, the telomerase activity was correlated with hTERT expression (positive *vs* negative expression of telomerase activity and hTERT, P=0.021).

CONCLUSION: Telomerase activity is closely correlated with the occurrence, development and metastasis of colorectal carcinoma. Overexpression of hTERT may play a critical role in the regulation of telomerase activity.

Key words: Colorectal carcinoma; Telomerase activity; hTERT expression

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INTRODUCTION

Colorectal carcinoma is one of the most common malignant tumors in digestive system, threatening human life and health. Recent investigations demonstrated that the telomerase activity is significantly increased in human malignant tumors, but is not expressed in normal somatic cells[1,2], suggesting there is a close relation between telomerase activity and malignant tumors. In order to study the role of telomerase activity in the carcinogenesis and progression of colorectal carcinoma as well as the correlation between telomerase activity and hTERT expression, the telomerase activity and hTERT expression were determined in colorectal carcinoma and its adjacent tissues, normal mucosa and adenomatoid polyp in this study.

MATERIALS AND METHODS

Patients and specimens

Thirty specimens of colorectal carcinoma and corresponding paracancerous tissues and normal mucosa were obtained by surgical resection, 20 specimens of adenomatoid polyp were taken during endoscopic examination. All patients were diagnosed pathologically and no patient received radiotherapy or chemotherapy before the sampling. Clinical staging showed Dukes' A in none, Dukes' B in 2 cases, Dukes' C in 24 cases, and Dukes' D in 4 cases. Well-differentiated adenocarcinoma

Table 1 Telomerase activity and hTERT expression in four different groups

Group	*n*	Telomerase *n* (%)	hTERT *n* (%)
Colorectal carcinoma	30	25 (83.33)[a]	23 (76.67)[a]
Adjacent peritumoral tissues	30	4 (13.33)	5 (16.67)
Adenomatoid polyp	20	2 (10.00)	2 (10.00)
Normal mucosa	30	1 (3.33)	1 (3.33)

[a]$P<0.05$ ($\chi^2=59.58$, $\chi^2=49.23$) *vs* adjacent peritumoral tissues, adenomatoid polyp and normal mucosa.

Table 2 Relation between telomerase activity and hTERT expression and clinical pathologic factors of colorectal carcinoma

Group	*n*	Telomerase (%)	hTRET (%)
Dukes' A	0	0	0
Dukes' B	2	1/2[a]	1/2[a]
Dukes' C	24	83.33 (20/24)	83.33 (20/24)
Dukes' D	4	4/4[a]	2/4[a]
High differentiation	5	2/5[a]	3/5[a]
Moderate differentiation	8	6/8[a]	5/8[a]
Poor differentiation	17	100.00 (17/17)	88.24 (15/17)
With lymphatic metastasis	10	80.00 (8/10)[b]	70.00 (7/10)[b]
Without lymphatic metastasis	20	0.00 (0/20)	5.00 (1/20)

[a]indicates cases less than 10 not included in the percentage; [b]$P<0.01$ *vs* the group without lymphatic metastasis

was found in 5 cases, moderately-differentiated adenocarcinoma in 8 cases and poorly-differentiated adenocarcinoma in 17 cases. Ten patients had lymph node involvement and 20 had no lymph node involvement. Of the patients with adenomatoid polyp, two had accompanying moderate-severe atypical hyperplasia. Specimens were collected within 30 minutes *in vitro*. Each specimen was divided into two parts, one for pathological diagnosis and immunohistochemical staining and the other for telomerase activity assay.

TRAP PCR ELISA protocol for telomerase activity assay

Telomerase activity was detected with a kit (Roche, Germany). Primers TS (5' AATCCGTCGAGCAGAGTT) and CX (5' CCCTTACCCTTACCCTTACCCTAA) were designed. Thirty cycles of PCR were performed at 25℃ for 30 min, at 94℃ for 5 min, at 94℃ for 30s, at 50℃ for 30s, at 72℃ for 90s, and a final extension at 72℃ for 10 min. The PCR products were analyzed and defined positive when $A>0.2$ on the reading of the microplate reader.

hTERT expression detection by immunohistochemical staining

Expression of hTERT was detected by immunohistochemical assay with the kit provided by Beijing Zhongshan Golden Biotechnology Co. Ltd according to the manufacturer's instructions. S-P method was adopted and positive calls were calculated and compared according to the accessory tester of the product. A negative control was prepared for each sample using PBS as the primary antibody. Microscopically, no staining was negative, karyon and perikaryon cytoplasms with brown granules were defined as positive cells.

Table 3 Correlation between telomerase activity and hTERT expression

		hTERT		
Telomerase	*n*	+	--	*P*
+	25	20	5	<0.05
--	5	3	2	

Statistical analysis

Statistical analyses were carried out with PEMS statistical-software. Chi-square test or Fisher's exact test was used for data processing. $P<0.05$ was considered statistically significant.

RESULTS

Telomerase activity and hTERT expression in colorectal carcinoma and its adjacent tissues, normal mucosa and adenomatoid polyp

The high telomerase activity and hTERT expression were found in 25 (83.33%) and 23 (76.67%) out of the 30 specimens of colorectal carcinoma, but in only 4 (13.33%) and 5 (16.67%) specimens of adjacent peritumor tissues, in 1 (3.33%) and 1 (3.33%) specimens of normal mucosa, in 2 (10.00%) and 2 (10.00%) out of the 20 specimens of adenomatoid polyp (Table 1).

Relation between telomerase activities and hTERT expression and clinical pathologic factors of colorectal carcinoma

The telomerase activity and hTERT expression had a close relation with lymphatic metastasis, and the positive expression rate in the patients with lymphatic metastasis was significantly higher than that in the patients without lymphatic metastasis ($P<0.05$). The telomerase activity and hTERT expression increased when the histological grade and clinical staging were greater, but the difference was of no statistical significance ($P>0.05$, Table 2).

Relation between telomerase activity and hTERT expression

The expression coincidence rate of telomerase activity and hTERT expression in colorectal carcinoma and its adjacent tissues, normal mucosa and adenomatoid polyp was 92% (23/25), 80% (4/5), 100%(1/1), 100%(2/2) respectively. The total coincidence rate of telomerase activity and hTERT expression was 90.91% (30/33). Of the 30 cases of colorectal carcinoma, 20 cases had positive expression in telomerase activity and hTERT expression, 2 showed no telomerase activity and hTERT expression. The conformity rate was 73.33% (22/30) ($P<0.05$, Table 3).

DISCUSSION

Telomerase is a ribonucleoprotein complex (a cellular

reverse transcriptase) consisting of three components: human telomerase RNA (hTR), telomerase-associated protein 1 (TP1/TLP) and human telomerase reverse transcriptase (hTERT). The first two components are expressed constitutively in both normal and tumor tissues and their expression levels are not correlated with the telomerase activity, whereas hTERT expression is closely correlated with telomerase activity in cells and tissues. Telomerase uses its internal RNA component as a template to synthesize telomeric DNA (TTAGGG)n, participating in the maintenance of telomere length and immortalization of cells[3]. Shay *et al*[4] reported that the total positive rate of telomerase activity was 85% in more than 20 malignant tumors and merely 9% in adjacent peritumor tissues and normal tissues, suggesting that telomerase activity is closely associated with malignancies. Our study showed that the telomerase activity and hTERT expression in colorectal carcinoma (83.33% and 76.67% respectively) were much higher than those in adjacent peritumoral tissues, normal mucosa and adenomatoid polyp. The telomerase activity and hTERT expression were positive in 2 cases of adenomatoid polyp with a diameter >2 cm, suggesting that telomerase activity may be an important index of canceration. This result is consistent with the findings of Tang *et al*[5]. The telomerase activity was higher in colorectal carcinoma with lymphatic metastasis than in that without lymphatic metastasis ($P<0.01$), indicating that the level of telomerase activity is closely related with lymphatic metastasis. The increased telomerase activity was in accord with the histological grade and staging of tumor, suggesting that telomerase activity plays a key role in the occurrence and development of colorectal carcinoma, and can be used as a marker for the early diagnosis and prognostic estimation of colorectal carcinoma.

hTERT is a telomerase reverse transcriptase isoform which is highly expressed in cell lines of positive telomerase[4]. hTERT transcription translated with hTR is indicative of telomerase activity *in vitro*. The activity of telomerase decreases or even disappears if the amino acid in hTERT is changed, whereas introduction of hTERT into normal cells activates the telomerase and prolongs cell life span while the karyotype and phenotype of cells remain normal[6-9]. In our study, the expression of hTERT was closely associated with telomerase activity, the coincidence rate of telomerase activity and hTERT expression was as high as 90.91% (30/33), suggesting that telomerase activity is related with hTERT expression. The results support the opinion that hTERT is an importent rate-limiting determinant of telomerase activity and the expression level of hTERT is directly associated with the telomerase activity. Since hTERT test can be conducted in paraffin- embedded tissues, more samples are available for telomerase activity test and hTERT may be used as a new tumor marker.

In conclusion, telomerase activity and hTERT expression in colorectal carcinoma are closely related. hTERT may play an important role both in the activation of telomerase and in the formation and development of colorectal carcinoma. hTERT can be used as a new marker for the early diagnosis of colorectal carcinoma.

REFERENCES

1 **Ramakrishnan S**, Eppenberger U, Mueller H, Shinkai Y, Narayanan R. Expression profile of the putative catalytic subunit of the telomerase gene. *Cancer Res* 1998; **58**: 622-625

2 **Urquidi V**, Tarin D, Goodison S. Role of telomerase in cell senescence and oncogenesis. *Annu Rev Med* 2000; **51**: 65-79

3 **Meyerson M**, Counter CM, Eaton EN, Ellisen LW, Steiner P, Caddle SD, Ziaugra L, Beijersbergen RL, Davidoff MJ, Liu Q, Bacchetti S, Haber DA, Weinberg RA. hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. *Cell* 1997; **90**: 785-795

4 **Shay JW**, Bacchetti S. A survey of telomerase activity in human cancer. *Eur J Cancer* 1997; **33**: 787-791

5 **Tang R**, Cheng AJ, Wang JY, Wang TC. Close correlation between telomerase expression and adenomatous polyp progression in multistep colorectal carcinogenesis. *Cancer Res* 1998; **58**: 4052-4054

6 **Weinrich SL**, Pruzan R, Ma L, Ouellette M, Tesmer VM, Holt SE, Bodnar AG, Lichtsteiner S, Kim NW, Trager JB, Taylor RD, Carlos R, Andrews WH, Wright WE, Shay JW, Harley CB, Morin GB. Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT. *Nat Genet* 1997; **17**: 498-502

7 **Beattie TL**, Zhou W, Robinson MO, Harrington L. Reconstitution of human telomerase activity in vitro. *Curr Biol* 1998; **8**: 177-180

8 **Nakayama J**, Tahara H, Tahara E, Saito M, Ito K, Nakamura H, Nakanishi T, Tahara E, Ide T, Ishikawa F. Telomerase activation by hTRT in human normal fibroblasts and hepatocellular carcinomas. *Nat Genet* 1998; **18**: 65-68

9 **Bodnar AG**, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE. Extension of life-span by introduction of telomerase into normal human cells. *Science* 1998; **279**: 349-352

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RAPID COMMUNICATION

Helicobacter pylori infection in the pharynx of patients with chronic pharyngitis detected with TDI-FP and modified Giemsa stain

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Abstract

AIM: To detect whether there is *Helicobacter pylori (H pylori)* colonization in the pharynx mucous membrane of healthy people and whether chronic pharyngitis is related to *H pylori* infection.

METHODS: Fifty cases of chronic pharyngitis refractory over three months were prospectively studied from March 2004 to August 2004 in the otolaryngology outpatient department of the Second Hospital of Xi'an Jiaotong University. Template-directed dye-terminator incorporated with fluorescence polarization detection (TDI-FP) and modified Giemsa stain were used to examine pharynx mucous membrane tissue for *H pylori* colonization in the patients with chronic pharyngitis and the healthy people as a control group.

RESULTS: In the control group, no people were detected to have *H pylori* in the pharynx. In contrast, in 50 cases with chronic pharyngitis, 19 (38.0%) cases were *H pylori* positive with a TDI-FP assay and 4 (8%) cases were TDI-FP positive with Giemsa staining in the pharynx. Sixteen of the 50 pharyngitis cases had stomach ailment history, 11 cases (68.8%) of these 16 patients were determined to be *H pylori* positive in the pharynx with the TDI-FP assay. χ^2 test showed that this infection rate was remarkably higher ($P = 0.0007$) than that in the cases without stomach ailment history. Giemsa staining showed that 3 cases (18.8%) of the patients with stomach ailment history were infected with *H pylori* in the pharynx, which was remarkably higher ($P = 0.042$) than that in the patients without stomach ailment history (1 case, which was 2.9%).

CONCLUSION: *H pylori* may not be detected in the pharynx of healthy people. Chronic pharyngitis may be related to *H pylori* infection. The infection rate with *H pylori* in the pharynx is higher in patients with stomach ailment histories than in patients without stomach ailment histories, suggesting that chronic pharyngitis may be related to stomach ailment history.

Key words: Chronic pharyngitis; *H pylori*; Modified Giemsa stain

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INTRODUCTION

Chronic pharyngitis is a common disease in the otolaryngology clinic. Pharyngitis, bronchitis, and pneumonia represent the most common respiratory tract infections. Upper respiratory tract infections are common and important. These include sinusitis, otitis media, and pharyngitis/tonsillitis. Many patients visiting an office-based physician report "sore throat, foreign-body sensation in the throat" as their primary reason for the visit[1]. Acute pharyngitis may be caused by a wide variety of microbial agents, but some of the most common and potentially dangerous microorganisms are group A beta-hemolytic streptococci (GABHS). For example, streptococcal pharyngitis is responsible for about 5% to 17% of sore throats in adults. However, the causative microorganisms in many cases remain unclear[2]. An important risk factor for chronic pharyngitis is gastroesophageal reflux disease (GERD). GERD is a common disorder in the Western population and is also common in Xi'an's adult population. The etiology and pathogenesis of GERD are probably associated with other conditions that are also risk factors

for chronic pharyngitis, including functional dyspepsia (FD), irritable bowel syndrome (IBS), and some respiratory and laryngopharyngeal diseases[3].

The Gram-negative bacterium *Helicobacter pylori* (*H pylori)* is associated with severe gastric pathologies, including peptic ulcers, chronic active gastritis and gastric cancer. This microorganism is able to invade and colonize in the human stomach, gastric juice, saliva, and faeces of patients[4-6]. Moreover, as relates to the current study, investigations using the CLO (Campylobacter-like organism) test and PCR showed that there was a high rate of *H pylori* colonization in tonsil and adenoid tissues[7,8], however, another investigation indicated the opposite result [9]. Therefore, we sought to detect whether there was *H pylori* colonization in the pharynx in healthy people, as compared to patients suffering from chronic pharyngitis. We used two methods to detect *H pylori* infection in the pharynx: template-directed dye-terminator incorporated with fluorescence polarization detection (TDI-FP) and modified Giemsa stain. From this study, it can be determined more definitively whether there is a relationship between *H pylori* infection of the pharynx and chronic pharyngitis; moreover, it can be determined if a history of certain gastric diseases may contribute to this infection.

MATERIALS AND METHODS

Patients

Two groups were studied, a group with chronic pharyngitis and a control group without chronic pharyngitis. The group with chronic pharyngitis consisted of 50 cases of chronic pharyngitis refractory over three months that were studied from March, 2004 to August, 2004 in the otolaryngology outpatient department. Among them, 32 cases were females and 18 cases were males, with ages ranging from 23 to 52 years old. The shortest disease history was 3 mo and the longest was 8 years. Major symptoms included foreign body sensation, dry sensation, angina in the pharynx, nausea, cough and belching. Clinical examination showed congestion in the pharynx mucosa, lymph follicle hyperplasia, and slightly swollen tonsils. Of these 50 cases, there were 34 cases with no specific stomach ailment history and 16 cases with gastric ulcers or chronic active gastritis. There were 20 cases in the control group. Of these, 12 cases were females and 8 cases were males. Ages ranged from 20-51 years old. The people in the control group had no specific pharyngitis or stomach ailment history, such as gastric ulcer or chronic active gastritis. In addition, all the people in the above two groups (1) had no other system diseases; (2) had no antibiotics treatment 10 d before examination; and (3) had no clear history of metronidazole, amoxicillin or tetracycline treatment. To collect tissue from the pharynx of each patient, the surface of the pharynx mucous membrane was sprayed with 20 g/L amethocaine for anaesthesia. Epithelial tissue in the pharynx was then collected with a sterilized curette. The protocol was approved by the Institutional Human Subject Committee at Xi'an Jiaotong University.

Methods

The biopsied epithelial tissue from the pharynx of each patient was routinely fixed in 4 g/L formaldehyde and embedded in paraffin wax. Histological sections were stained with routine modified Giemsa.

Fluorescence Polarization-Capable Instrument-Victor, AmpliTaq DNA Polymerase, shrimp alkaline phosphatase, *E. coli* exonuclease I, mixtures of TAMRA-ddTTP and RP110-ddGTP were purchased from PerkinElmer. The pGEM-T-Easy Vector System TA Cloning Kit was purchased from Promega. The ABI 377 and BigDye Terminator Cycle Sequencing Kit were purchased from Applied Biosystem(s). All reactions were run and read in 96-well black-skirted plates purchased from MJ Research. The specific probes and terminators of *H pylori* were designed by DNA Star based on a specific target sequence. All probes were synthesized by Sbsbio (Beijing, China).

Scrapes or biopsies were collected and washed into 5 mL PBS (pH 7.2). In each case, the cell suspension was centrifuged for 5 min at 10 000 r/min. The cell pellet was re-suspended and mixed in 1.5 mL TE buffer (200 mg/L proteinase K, 10 mmol/L Tris, 0.1 mmol/L EDTA, 1 g/L SDS, 3 g/L Triton X-100) at 37°C for 12 h. The suspension was incubated at 95°C for 10 min to inactivate proteinase K, and then it was centrifuged for 5 min at 10 000 r/min. The supernatant DNA was used as a template for PCR.

Amplification of H pylori DNA by using a conservative primer pair

For extensive detection of *H pylori* DNA, each sample DNA was first amplified in a 25 μL reaction mixture containing 1 μL of DNA extract, 2 μL of 6.25 pmol/L common conservative primer pair (P1, 5' tgcccgcttc-cactaaccccacta 3'; P2, 5' gtcagccactttgcccacttctacag 3', *H pylori* urease B (*ureB*) gene (gene bank accession number AY295085), 10×PCR reaction buffer (10 mmol/L Tris-HCl, 50 mmol/L KCl), 2.5 μL of 2.5 mmol/L dNTP, 1.5 μL of 25 mmol/L $MgCl_2$, and 2.5 μL of 0.25 U AmpliTaq DNA Polymerase. The mixture was denatured initially for 5 min at 94°C, followed by 30 cycles of amplification in a PCR processor. Each cycle included a denaturing step of 94°C for 1 min, an annealing step of 45°C for 1 min and a chain elongation step of 72°C for 1.5 min. The final elongation step was then prolonged for another 5 min. The size of all *H pylori* genotyping PCR products was predicted to be 413bp according to gene bank accession number AY295085. Sample containing other plasmid DNA was the negative control.

All PCR products were analyzed using a template directed dye-terminator incorporation assay (TDI) with fluorescence polarization (FP). In order to degrade excess dNTP and common conservative primers in the PCR product, 1μL of PCR product, 16.67 nkat of shrimp alkaline phosphatase, 1U of *E. coli* exonuclease I in 1 μL of shrimp alkaline phosphatase buffer (0.5 mol/L Tris-HCl, pH8.5, 50 mmol/L $MgCl_2$), and 8 μL of distilled water were mixed and incubated at 37°C for 60 min before enzymes were heat-inactivated at 80°C for 15 min. Thirteen microliters of TDI-FP mixture containing 10× reaction

Table 1 *H pylori* infection in the pharynx of patients with chronic pharyngitis

Group	Case	TDI-FP(%)	Giemsa(%)
Chronic pharyngitis	50	19 (38.0)[a]	4 (8.0)
With stomach ailment history	16	11 (68.8)[b]	3 (18.8)[b]
Without stomach ailment history	34	8 (23.5)	1 (2.9)
Normal (Control)	20	0	0

[a]$P \leqslant 0.05$ *vs* Control; [b]$P \leqslant 0.01$ *vs* group without stomach ailment history.

buffer, TAMRA-ddTTP, RP110-ddGTP, *H pylori* probes and 7 μL of the enzymatically treated PCR product were mixed and denatured at 95 °C for 2 min, followed by 25 cycles of 95 °C for 15 s and 50 °C for 30 s. At the end of the cycles, the mixture was held at room temperature. *H pylori* genes were acquired by reading FP (mp) at wavelengths of 535 nm and 595 nm, and analyzed by Fluorescence Polarization-Capable Instrument-Victor.

Statistical analysis

All the data were analyzed by the χ^2 test.

RESULTS

H pylori infections in the pharynx of the people in the control group and the patients suffering from chronic pharyngitis were examined with TDI-FP and modified Giemsa stain. In the control group, analysis with TDI-FP showed that none of the 20 cases was infected with *H pylori* in the pharynx. The examination using modified Giemsa stain revealed that no cases were infected with *H pylori* in the pharynx.

Regarding the 50 cases of the patients who were suffering from chronic pharyngitis, 19 cases (38%) were determined using TDI-FP to be infected with *H pylori* in the pharynx. When the modified Giemsa stain procedure was used, 4 cases (8.0%) of patients were determined to be infected with *H pylori* in the pharynx. In the 16 pharyngitis cases with stomach ailment history, 11 cases (68.8%) were determined using TDI-FP to be infected with *H pylori* in the pharynx, which was remarkably higher ($P \leqslant 0.01$) than that in the cases without stomach ailment history (8 cases, 23.5%) as determined by the Statistical χ^2 test. From using the modified Giemsa stain method, 3 cases (18.8%) of patients with a stomach ailment history were determined to be infected with *H pylori* in the pharynx, which was remarkably higher (P10 $\leqslant$ 0.01) than that in patients without a stomach ailment history (1 case, 2.9%), as determined by the statistical χ^2 test (Table 1). This showed the following: (1) that *H pylori* is not detected in the pharynx of healthy people; (2) that chronic pharyngitis is often related to *H pylori* infection; and (3) that a stomach ailment history is associated with a higher rate of *H pylori* infection of the pharynx.

DISCUSSION

We used two methods to detect *H pylori* infection, template-directed dye-terminator incorporation with fluorescence polarization (TDI-FP), and modified Giemsa stain. TDI-FP is a single base extension technique in which an oligonucleotide probe anneals to a polymerase chain reaction (PCR)-amplified product adjacent to a single nucleotide polymorphism (SNP) of interest[3,10]. In the presence of DNA polymerase and fluorescently labeled dideoxyribonucleoside triphosphates (ddNTPs), the probe is extended by a single base dictated by the polymorphic site in the target sequence. Fluorescence polarization, the property that fluorescent molecules emit polarized fluorescent light when excited by plane polarized light, is used to identify incorporated ddNTP, and to assign a genotype[10]. With this design, the PCR primers will not interfere with the primer extension step of the assay. This method is simple, rapid, sensitive, specific and could also be used for detecting pathogen DNA in the clinic[11,12]. The modified Giemsa stain is very straightforward, inexpensive, and takes about five minutes to perform, excluding the time in solution, and rarely requires repeat stains[13].

In the current study, we examined for *H pylori* infection in the pharynx with TDI-FP and the modified Giemsa stain methods. These two methods are rapid, sensitive, specific, easily reproducible and easy to perform technically. The secretion in the pharynx in our study was washed out by water from the epithelial tissue in the checked pharynx before examination so that the results were not affected by gastric juice and saliva.

Sore throat is one of the most common reasons for visits to family physicians. While most patients with sore throat have an infectious cause (such as pharyngitis), fewer than 20 percent have a clear indication for antibiotic therapy (i.e., group A beta-hemolytic streptococcal infection). Useful, well-validated clinical decision rules are available to help family physicians care for patients who present with pharyngitis[14]. Because of recent improvements in rapid streptococcal antigen tests, throat culture can be reserved for patients whose symptoms do not improve over time or who do not respond to antibiotics. Pharyngitis and the common cold are among the most frequent diseases. Two thirds of the patients consulting for respiratory infection received antibiotic treatment, and antibiotics confer relative benefits in the treatment of sore throat[16]. Pharyngitis may be caused by a wide variety of microbial agents. *Burkholderia cepacia* is a Gram-negative bacillus that is widely distributed in nature; pharyngitis due to *Burkholderia cepacia* was reported for person-to-person transmission[17]. The main aetiological agents reportedly causing acute pharyngitis were adenovirus, respiratory syncytial virus (RSV), *Mycoplasma pneumoniae*, *Streptococcus pyogenes* and *Chlamydia pneumoniae*. *M. pneumoniae* was the agent found most frequently as a single pathogen. A history of recurrent pharyngitis, having older siblings and a negative outcome were significantly more common among patients with acute *M. pneumoniae* infection than among those with infections due to other pathogens, or healthy controls. That study demonstrates that: (1) adenovirus and RSV have a prominent role in acute pharyngitis; (2) *S. pyogenes* is found frequently, but it is not possible to distinguish simple carriers from patients with a true infection; and (3) *M. pneumoniae* appears to be

able to cause acute pharyngitis[18]. In our daily work, we use antibiotics to treat pharyngitis and sore throat and find antibiotics are effective, although we are not clear about the mechanism of the treatment.

H pylori is one of the world's most widespread microorganisms. The abrupt increase of *H pylori* during high school may result from a marked increase of interpersonal social activities[19]. Its acquisition in humans remains poorly understood, however, epidemiological studies have identified drinking water as a reservoir for the bacterium[20]. *H pylori* has been associated with the development of gastritis, peptic ulcers and gastric cancer. Although *H pylori* infects up to more than half of the world's population, to date the precise modes of transmission have not been fully understood[13]. *H pylori* has been investigated in several other organ systems and localizations besides the gastrointestinal cavity, such as the oral cavity. For example, in one study, it was found that the majority of patients with oral diseases have possible *H pylori* colonization in dental plaque; while about two-thirds have *H pylori* associated chronic active gastritis. The oral cavity may be the first place for colonization by *H pylori*, and then the infection involves the gastric mucosa[21]. Oral hygiene (the frequency of dental visits and teeth cleaning) did not have a significant influence on the presence of *H pylori* in dental plaque. Other investigators supported the hypothesis that *H pylori* infection begins in the oral cavity by concluding that dental plaque is the reservoir of *H pylori* with no relationship to gastric infection[22]. In our study, the pharynx may be a place for *H pylori* colonization, but the number of *H pylori* was so few that the modified Giemsa stain was not very useful. Therefore, we made an amplification of *H pylori* DNA by using a conservative primer pair, then detected the *H pylori* colonization using TDI-FP and acquired a good result.

Other investigators have studied the localization of *H pylori* as well. Akbayir *et al*[23] demonstrated that *H pylori* was not present in laryngeal squamous cell carcinoma tissue or in benign lesions. They could not find any evidence indicating that *H pylori* played a role at the tissue level in the pathogenesis of laryngeal carcinoma. Their results may indicate that *H pylori* does not colonize in either adenoid or tonsils and that these tissues do not constitute a reservoir for *H pylori* infection. On the contrary, Cirak *et al*[7] detected *H pylori* and its *CagA* gene in tonsil and adenoid tissues by PCR. These authors found: 7 of 23 (30%) patients to be positive for *H pylori* DNA, 5 (71%) of whom also possessed the *CagA* gene. In another study, specimens from 208 dyspeptic patients were collected from saliva, supra- and subgingival dental plaque, tongue scrapings, and oropharyngeal swabs. *H pylori* was detected from multiple sites (dental plaque, gastric juice, gastric biopsy, duodenal aspirate, and the oropharynx[26]). As in our study, the authors used more than one method for detecting *H pylori*. When PCR was used, 15 of 208 patients (7%) tested positively for *H pylori* by PCR in dental plaque. In contrast, only 2 samples were positive by culture. This demonstrates the importance of using more than one method for detecting *H pylori* infection. We also found this to be true in our own study, where we used two methods, TDI-FP and modified Giemsa stain, to detect *H pylori* infection. As shown in Table 1, TDI-FP consistently detected *H pylori* infections that were missed by the modified Giemsa stain method. These authors also found that four of the dental plaque strains had restriction patterns similar to those of the stomach and duodenal sites, providing evidence that these sites were infected with the same strain of *H pylori*. The detection in dental plaque could indicate that the oral cavity may act as a reservoir or sanctuary for the organism. Likewise, this finding also relates to our data. We found that *H pylori* infection in the pharynx was significantly more likely in patients with a history of stomach ailments than in patients without a history of stomach ailments. That is, just as there is a relationship between the occurrence of *H pylori* in dental plaque and its occurrence in the stomach and duodenum, there may be a relationship between infection of the pharynx with *H pylori* and a history of stomach ailments. However, whether *H pylori* is a resident or transient oral microorganism is still unclear, although it is more likely to be transient in nature[27].

In our study, the goal was to determine if there was a relationship between *H pylori* infection in the pharynx and chronic pharyngitis, and, in addition, to determine if such infections correlated with a history of stomach ailments. We determined both of these relationships to be true. In addition, we utilized two methods for detecting *H pylori*, TDI-FP and modified Giemsa stain, and found the TDI-FP method to be the most sensitive. These findings can be applied to the clinical setting: doctors will be able to use the appropriate method to test if a patient with chronic pharyngitis is infected in the pharynx with *H pylori*, aiding in prescribing the appropriate antibiotic. These findings also should cause doctors to examine a patient with chronic pharyngitis for ailments in the digestive system. But on the other hand, the TDI-FP test can yield false positive results.

To determine whether *H pylori* infection is related to other upper respiratory tract infections, such as sinusitis, or with otitis media, further investigations will need to be performed.

REFERENCES

1 **Schwartz K**, Monsur J, Northrup J, West P, Neale AV. Pharyngitis clinical prediction rules: effect of interobserver agreement: a MetroNet study. *J Clin Epidemiol* 2004; **57**: 142-146
2 **Merrill B**, Kelsberg G, Jankowski TA, Danis P. Clinical inquiries. What is the most effective diagnostic evaluation of streptococcal pharyngitis? *J Fam Pract* 2004; **53**: 734, 737-78, 740
3 **Wang JH**, Luo JY, Dong L, Gong J, Tong M. Epidemiology of gastroesophageal reflux disease: a general population-based study in Xi'an of Northwest China. *World J Gastroenterol* 2004; **10**: 1647-1651
4 **De Luca A**, Iaquinto G. Helicobacter pylori and gastric diseases: a dangerous association. *Cancer Lett* 2004; **213**: 1-10
5 **Zhang C**, Yamada N, Wu YL, Wen M, Matsuhisa T, Matsukura N. Helicobacter pylori infection, glandular atrophy and intestinal metaplasia in superficial gastritis, gastric erosion, erosive gastritis, gastric ulcer and early gastric cancer. *World J Gastroenterol* 2005; **11**: 791-796
6 **Zhang J**, Chen XL, Wang KM, Guo XD, Zuo AL, Gong J. Relationship of gastric Helicobacter pylori infection to Barrett' s esophagus and gastro-esophageal reflux disease in Chinese. *World J Gastroenterol* 2004; **10**: 672-675

7 **Cirak MY**, Ozdek A, Yilmaz D, Bayiz U, Samim E, Turet S. Detection of Helicobacter pylori and its CagA gene in tonsil and adenoid tissues by PCR. *Arch Otolaryngol Head Neck Surg* 2003; **129**: 1225-1229

8 **Unver S**, Kubilay U, Sezen OS, Coskuner T. Investigation of Helicobacter pylori colonization in adenotonsillectomy specimens by means of the CLO test. *Laryngoscope* 2001; **111**: 2183-2186

9 **Yilmaz M**, Kara CO, Kaleli I, Demir M, Tümkaya F, Büke AS, Topuz B. Are tonsils a reservoir for Helicobacter pylori infection in children? *Int J Pediatr Otorhinolaryngol* 2004; **68**: 307-310

10 **Freeman BD**, Buchman TG, Zehnbauer BA. Template-directed dye-terminator incorporation with fluorescence polarization detection for analysis of single nucleotide polymorphisms associated with cardiovascular and thromboembolic disease. *Thromb Res* 2003; **111**: 373-379

11 **Guo YH**, Zhang J, Zhao JR. Dectecting HBV DNA in serum by PCR-FP. *Chin J Lab Med* 2004; **27**: 26-28

12 **Bai YJ**, Zhao JR, Lv GT, Zhang WH, Wang Y, Yan XJ. Rapid and high throughput detection of HBV YMDD mutants with fluorescence polarization. *World J Gastroenterol* 2003; **9**: 2344-2347

13 **Rotimi O**, Cairns A, Gray S, Moayyedi P, Dixon MF. Histological identification of Helicobacter pylori: comparison of staining methods. *J Clin Pathol* 2000; **53**: 756-759

14 **Leong RW**, Lee CC, Ling TK, Leung WK, Sung JJ. Evaluation of the string test for the detection of Helicobacter pylori. *World J Gastroenterol* 2003; **9**: 309-311

15 **Vincent MT**, Celestin N, Hussain AN. Pharyngitis. *Am Fam Physician* 2004; **69**: 1465-1470

16 **Romero Vivas J**, Rubio Alonso M, Corral O, Pacheco S, Agudo E, Picazo JJ. [Community-acquired respiratory infections]. *Enferm Infecc Microbiol Clin* 1997; **15**: 289-298

17 **Fajardo Olivares M**, Cordero Carrasco JL, Beteta López A, Escobar Izquierdo AB, Sacristán Enciso B. [Pharyngitis due to Burkholderia cepacia. Person-to-person transmission]. *An Pediatr (Barc)* 2004; **60**: 581-582

18 **Esposito S**, Blasi F, Bosis S, Droghetti R, Faelli N, Lastrico A, Principi N. Aetiology of acute pharyngitis: the role of atypical bacteria. *J Med Microbiol* 2004; **53**: 645-651

19 **Wu TC**, Chen LK, Hwang SJ. Seroprevalence of Helicobacter pylori in school-aged Chinese in Taipei City and relationship between ABO blood groups. *World J Gastroenterol* 2003; **9**: 1752-1755

20 **Rolle-Kampczyk UE**, Fritz GJ, Diez U, Lehmann I, Richter M, Herbarth O. Well water--one source of Helicobacter pylori colonization. *Int J Hyg Environ Health* 2004; **207**: 363-368

21 **Siddiq M**, Haseeb-ur-Rehman A. Evidence of Helicobacter pylori infection in dental plaque and gastric mucosa. *J Coll Physicians Surg Pak* 2004; **14**: 205-207

22 **Nasrolahei M**, Maleki I, Emadian O. Helicobacter pylori colonization in dental plaque and gastric infection. *Rom J Gastroenterol* 2003; **12**: 293-296

23 **Akbayir N**, Başak T, Seven H, Sungun A, Erdem L. Investigation of Helicobacter pylori colonization in laryngeal neoplasia. *Eur Arch Otorhinolaryngol* 2005; **262**: 170-172

24 **Crowe SE**. Helicobacter infection, chronic inflammation, and the development of malignancy. *Curr Opin Gastroenterol* 2005; **21**: 32-38

25 **Kashiwagi H**. Ulcers and gastritis. *Endoscopy* 2005; **37**: 110-115

26 **Sano N**, Ohara S, Koike T, Sekine H, Imatani A, Kitagawa Y, Dairaku N, Iijima K, Kato K, Shimosegawa T. [Influence of urease activity of the oral cavity and oropharynx on 13C-urea breath test]. *Nihon Shokakibyo Gakkai Zasshi* 2004; **101**: 1302-1308

27 **Oshowo A**, Gillam D, Botha A, Tunio M, Holton J, Boulos P, Hobsley M. Helicobacter pylori: the mouth, stomach, and gut axis. *Ann Periodontol* 1998; **3**: 276-280

28 **Chen X**, Kwok PY. Template-directed dye-terminator incorporation (TDI) assay: a homogeneous DNA diagnostic method based on fluorescence resonance energy transfer. *Nucleic Acids Res* 1997; **25**: 347-353

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RAPID COMMUNICATION

Heat-shocked tumor cell lysate-pulsed dendritic cells induce effective anti-tumor immune response *in vivo*

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Abstract

AIM: To study whether heat-shocked tumor cells could enhance the effect of tumor cell lysate-pulsed dendritic cells (DCs) in evoking anti-tumor immune response *in vivo*.

METHODS: Mouse undifferentiated colon cancer cells (CT-26) were heated at 42 °C for 1 h and then frozen-thawed. The bone marrow-derived DCs pulsed with heat-shocked CT-26 cell lysate (HSCT-26 DCs) were recruited to immunize syngeneic naïve BALB/c mice. The cytotoxic activity of tumor specific cytotoxic T lymphocytes (CTLs) in mouse spleen was evaluated by IFN-enzyme-linked immunospot (ELISpot) and LDH release assay. The immunoprophylactic effects induced by HSCT-26 DCs in mouse colon cancer model were compared to those induced by single CT-26 cell lysate-pulsed DCs (CT-26 DCs) on tumor volume, peritoneal metastasis and survival time of the mice.

RESULTS: Heat-treated CT-26 cells showed a higher hsp70 protein expression. Heat-shocked CT-26 cell lysate pulsing elevated the co-stimulatory and MHC-II molecule expression of bone marrow-derived DCs as well as interleukin-12 p70 secretion. The IFN-γ secreting CTLs induced by HSCT-26 DCs were significantly more than those induced by CT-26 DCs ($P = 0.002$). The former CTLs' specific cytotoxic activity was higher than the latter CTLs' at a serial E/T ratio of 10:1, 20:1, and 40:1. Mouse colon cancer model showed that the tumor volume of HSCT-26 DC vaccination group was smaller than that of CT-26 DC vaccination group on tumor volume though there was no statistical difference between them (24 mm^3 *vs* 8 mm^3, $P = 0.480$). The median survival time of mice immunized with HSCT-26 DCs was longer than that of those immunized with CT-26 DCs (57 d *vs* 43 d, $P = 0.0384$).

CONCLUSION: Heat-shocked tumor cell lysate-pulsed DCs can evoke anti-tumor immune response *in vivo* effectively and serve as a novel DC-based tumor vaccine.

Key words: Heat shock; Tumor; Dendritic cell; Immune

Qiu J, Li GW, Sui YF, Song HP, Si SY, Ge W. Heat-shocked tumor cell lysate-pulsed dendritic cells induce effective anti-tumor immune response *in vivo*. *World J Gastroenterol* 2006; 12(3): 473-478

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INTRODUCTION

Dendritic cells (DCs) are the most potent antigen-presenting cells (APCs) mediating effective immune effects *in vitro* and *in vivo*[1,2]. DCs-based tumor vaccines have been recruited to prevent postoperative recurrence and metastasis of malignant tumors[3,4]. During the development of this approach, distinct methods have been attempted to enhance the effect of DC tumor vaccine in evoking tumor rejection response.

Heat shock protein (HSP), the molecule chaperon in cells, binds to the antigenic peptides and guides them to accurate folding, transporting, and conjugating to major histocompatibility complex (MHC) molecules[5]. Referring to the current knowledge of HSPs, it acts as chaperone peptides including antigenic peptides, interacts with antigen presenting cells through a receptor, stimulates antigen presenting cells to secrete inflammatory cytokines and mediates maturation of DCs. It was reported that heating could enhance the immunogenicity of tumor cells, which is ascribed to HSPs[6]. Furthermore, vaccination with the lysate of heated tumor cells can result in effective tumor rejection *in vivo*[7]. In the present study, we used heat-shocked tumor cells to elicit HSP expression. We assumed that pulsing with these HSP-rich tumor cell lysate might enhance the effect of DCs to stimulate stronger anti-tumor response than pulsing with tumor cell lysates. Because hyperthermal treatment is a standardized manipulation in

clinical practice of oncologic surgery, such a DC tumor vaccine is convenient to be prepared. We examined the specific CTL response induced by heat-shocked tumor cell lysate-pulsed DCs in naïve mice and further evaluated its immunoprophylactic effects in mouse colon cancer model.

MATERIALS AND METHODS

Mice and cell line

Six to eight-week-old female BALB/c mice were purchased from Laboratory Animal Research Center (LARC) of the Fourth Military Medical University (FMMU, Xi'an) and housed under pathogen-free conditions. All experiments involving the use of mice were performed in accordance with the protocols approved by LARC. CT26 is a carcinogen-induced undifferentiated colon adenocarcinoma cell line of BALB/c mice. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) containing 10% heat-inactivated fetal bovine serum (FBS; Sijiqin Biotech, Hangzhou), 100 IU/mL penicillin and 100 μg/mL streptomycin at 37 °C in an atmosphere containing 50 mL/L CO_2 and passaged every 2 d.

Heating and lysing CT-26 cells

CT-26 cells at 90% confluence were heated in 42 °C water bath for 1 h followed by recovering for 2 h at 37 °C in an atmosphere containing 50 mL/L CO_2. Cells were then digested by 0.02% trypsin and washed twice with PBS. After being enumerated and re-suspended in PBS at 1×10^6 cells/mL, CT-26 cells were frozen in liquid nitrogen for 10 min and then thawed thrice at 4 °C. The frozen-thawed resultants were centrifuged at 12 000 *g* for 15 min and the supernatant was preserved as tumor cell lysate at -80 °C.

Western blot assay

Equivalent protein samples were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, the separated proteins were transferred onto nitrocellulose (NC) membranes. Membranes were blocked with 5% non-fat milk in TBST (20 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl, and 0.05% Tween 20) for 2 h and incubated for 1 h at room temperature with hsp70 mAb (H5147, Sigma) diluted in TBST. After washing, membranes were incubated with horseradish peroxidase conjugated goat-anti-rabbit IgG (Boxter) diluted 1:400 in TBST at room temperature for 1 h. Detection was performed using the DAB detection system.

Generation and pulse of bone marrow-derived DCs

Bone marrow-derived DCs were generated as described by Lutz *et al*[8] with minor modifications. Briefly, 1×10^6 cells/mL erythrocyte-depleted mouse bone marrow cells from flushed marrow cavities were cultured in complete medium (CM) with 20 ng/mL recombinant mouse GM-CSF (PeproTech, Rocky Hill, NJ, USA) in 10-cm tissue culture dishes at 37 °C in an atmosphere containing 50 mL/L CO_2. On d 3, 5, and 7, respectively, half media were removed and centrifuged for 5 min at 1 500 r/min, the collected cells were resuspended in the same volume of fresh CM and replenished to original plates. The frozen-thawed tumor lysate was added to the DC culture systems on d 7 at a ratio of five DC equivalents to one tumor cell (i.e., 5:1) and incubated at 37 °C in an atmosphere containing 50 mL/L CO_2. After 48 h of incubation, non-adherent cells including DCs were harvested by gentle pipetting. DCs were enumerated by FACS (FACScan, Becton Dickinson) analysis through staining with PE anti-mouse CD11c Ab (N418, hamster IgG, Biolegend). The co-stimulatory and MHC-Ⅱ molecules were analyzed by staining with FITC anti-mouse I-A/I-E (m5/114.15.2, ratIgG2b, Biolegend) and FITC anti-mouse CD86 (B7-2, PO3, ratIgG2b, Biolegend). The corresponding labeled isotypes served as the controls. For further vaccination, DCs were washed twice, enumerated and resuspended in PBS at 5×10^6/mL.

ELISA

Following the protocol of mIL-12 p70 ELISA Ready-SET-Go kit (Ebioscience, San Diego, CA, USA), plates (NUNC Maxisorp) were pre-coated with capture antibody (clone C18.2) overnight at 4 °C and blocked at room temperature for 1 h. After being washed, 100 μL/well of mIL-12 p70 standard (8 pg-1 024 pg/mL at twofold serial dilutions) or 100 μL/well of samples was added to the appropriate wells and incubated at room temperature for 2 h. Wells were aspirated and washed, then 100 μL/well detection antibody (clone C17.8) was added and incubated at room temperature for 1 h. After being washed, 100 μL avidin-HRP was added and incubated at room temperature for 30 min. Plates were washed thoroughly and 100 μL/well of substrate solution was added. After being incubated at room temperature for 15 min, 50 μL of stop solution was added to each well. The plates were read. Data represented the value of 450 nm subtracted the value of 570 nm. All assays were performed in triplicate.

Immunization assay and colonic cancer inoculation

BALB/c mice were immunized with DC vaccine through tail-vein on d 0 and 7 respectively at the same dose of 5×10^5 DCs (100 μL). Each treatment group contained not less than 15 mice. Seven days after the second immunization, three mice of each group were killed and the spleens were taken to perform the ELISpot and cytotoxic assays. The remaining mice were anesthetized with 0.75 mg of sodium pentobarbital. Colonic cancer inoculation was performed as follows. In brief, a vertical midline incision was made and the cecum was exposed. Upon visualization, 1×10^5 CT-26 cells (50 μL) were injected into subserosa using a 30-gauge needle (Becton Dickinson) and 1 mL syringe. The midline incision was closed with a running suture. Fourteen days later, not less than five mice of each group were killed, the weight of the colon tumors was measured and the peritoneal metastases were checked. The remaining mice were fed to observe the tumor-bearing survival time. The end point of observation was selected when all the mice of any of the two teams were dead.

ELISpot analysis

The murine interferon-gamma ELISpot kit (Diaclone, France) was used to determine tumor-specific IFN-γ

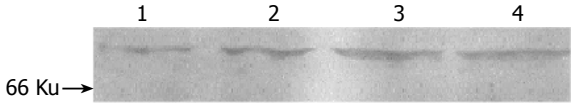

Figure 1 Western blot detection of HSP70 expression of CT-26 cells heating at 42 ℃. 1: No heating. 2: heat-shocked for 30 min. 3: heat-shocked for 1 h. 4: heat-shocked for 2 h.

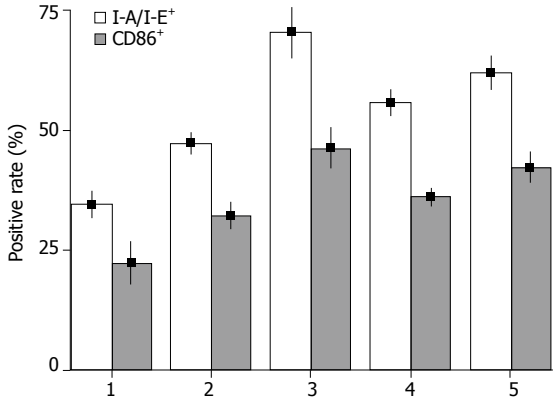

Figure 2 The surface molecule expressions of mouse bone marrow derived DCs were measured by staining with PE anti-mouse CD11c (N418, hamster IgG), FITC anti-mouse I-A/I-E (m5/114.15.2, ratIgG2b), FITC anti-mouse CD86 (B7.1, PO3, ratIgG2b) and analyzed by FACS. 1: DC culture system at d 7. 2: DC culture system at d 9. 3: DC culture system stimulating with LPS(1 μg/mL). 4: DC culture system pulsed with CT-26 lysates. 5: DC culture system pulsed with HSCT-26 lysates. The data are representative for 3 independent experiments.

secreting T cells[9]. The 96-well filtration plates (Nunc) were coated with 100 μL capture antibody (clone DB1). After an overnight incubation at 4 °C, the wells were washed and blocked with 2% dry skimmed milk in PBS. Splenocytes (1×10^6/well) isolated from the mice were added to the wells and incubated at 37 °C in an atmosphere containing 50 mL/L CO_2 for 20 h with target cells (5×10^4/well). Plates were washed and then incubated with 100 μL biotinylated detection antibody (polyclonal) at 37 °C for 90 min. After the removal of unbound antibodies, 100 μL avidin-alkaline phosphatase was added and plates were incubated for 1 h at 37 °C. After washing, spots were developed by adding 100 μL of ready-to-use BCIP/NBT buffer and incubated at room temperature for spot formation. The spots were scanned and counted.

Lactate dehydrogenase release assay

Specific cytolytic activities of murine spleen cells were determined by LDH assay. The CT-26 cells were used as target cells. Effector and target cells were mixed at the E/T ratio of 20:1 at 0.2 mL/well in 96-well round-bottomed plates (Nunc). After incubation for 4 h, cells were centrifuged at 250 r/min for 5 min and the cell-free supernatant was collected for LDH assay using CytoTox96 (Promega, Madison, WI, USA). The percentage of specific LDH release was calculated by the following formula: % cytotoxicity = [(experimental LDH release)-(spontaneous LDH release by effector and target cells)/(maximum LDH release)×(spontaneous LDH release)]×100. For the controls, the target cells were incubated either in culture medium alone to determine spontaneous release or in a mixture of 2% Triton X-100 to define the maximum LDH release. The spontaneous release was always <10% of the maximum release. All assays were performed in triplicate.

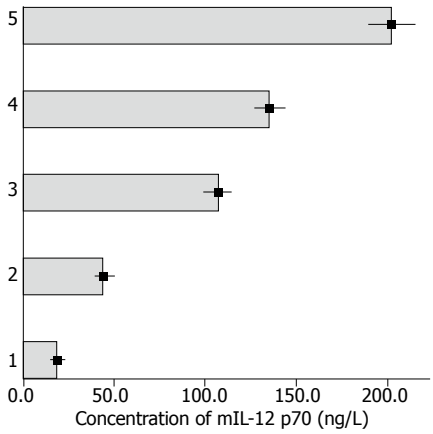

Figure 3 The concentration of mIL-12 p70 in supernatant of different DC culture system were measured by ELISA assay. 1: DC culture system at d 7. 2: DC culture system at d 9. 3: DC culture system pulsed with CT-26 lysates.4: DC culture system pulsed with HSCT-26 lysates. 5: DC culture system stimulating with LPS (1μg/mL). The data were representative for three independent experiments.

Statistical analysis

Each group included at least five mice. All experiments were carried out thrice. Data were expressed as mean ± SD. Tumor volume and immune cell yield data were analyzed by two-way analysis of variance (ANOVA). All analyses were conducted with SPSS8.0 software. $P<0.05$ was considered statistically significant.

RESULTS

Phenotype and cytokine production in heat-shocked CT-26 cell lysate-pulsed DCs

To evaluate the hsp70 protein expression in CT-26 cells, we performed Western blot analysis using an anti-hsp70 mAb. hsp70 protein in CT-26 cells increased after being heated for 30 min and peaked at 1 h (Figure 1). Therefore, heating at 42 °C for 1 h was selected as a standard treatment for heat shocking in the following experiment. To determine the quantity of DCs in culture system, we analyzed the surface molecules of harvested cells by flow cytometry. About 65% of the harvest cells in culture system on d 7 were CD11c positive and increased to 75% or more on d 9. DCs pulsed with HSCT-26 lysate manifested higher CD86 and I-A/I-E expression than pulsed with CT-26 lysate (Figure 2). We also measured the mIL-12 p70 concentration in the supernatant of different DC culture systems by ELISA. HSCT-26 lysate-pulsed DCs showed a higher level of mIL-12 p70 than either CT-26 lysate-pulsed or unpulsed DCs on d 9 (Figure 3).

Immunization with HSCT-26 lysate-pulsed DCs elicited tumor specific CTLs in naïve BALB/c mice

To investigate the ability of HSCT-26 lysate-pulsed DCs to induce CT-26 specific CTLs in naïve syngeneic BALB/c mice, we examined the CT-26 specific IFN-γ-producing T

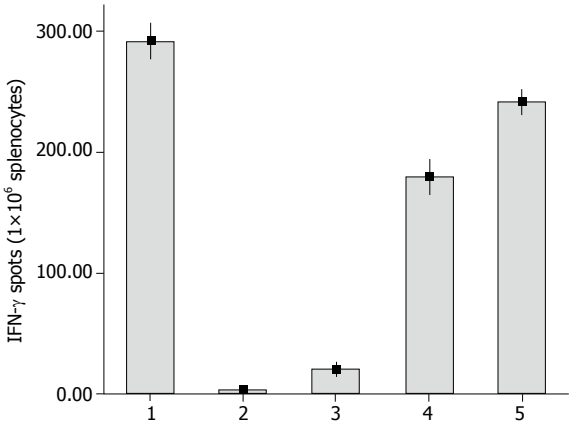

Figure 4 Tumor-specific IFN-γ secreting Splenic T cells induced by different cell lysates pulsed DC. 1: positive control (no target, 1mg/L PHA stimulated). 2: negative control (no target). 3: single DC immunized. 4:CT-26 lysates pulsed DC immunized. 5: HSCT-26 lysates pulsed DC immunized. Results are representative of three experiments.

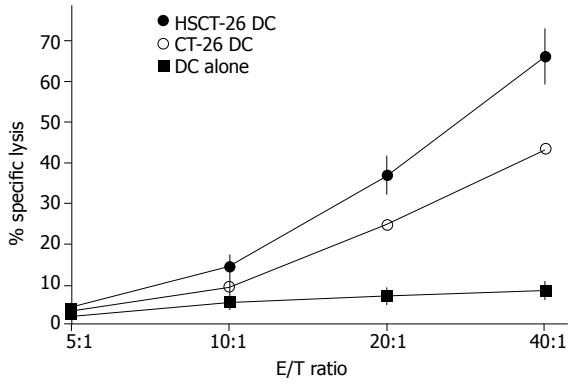

Figure 5 Bone marrow-derived DC pulsed with HSCT-26 lysate can generate tumor-specific CTLs *in vivo*. Splenic T cells obtained from immunized mice were evaluated for cytolytic activity against Mitomycin C treated CT-26 cells at the effector: target (E:T) ratios indicated. Values are the mean ± SE of triplicate wells.

cells in splenocytes derived from immunized and control mice by ELISpot assay. As shown in Figure 4, after being restimulated with mitomycin C-treated CT-26 cells at an E: T ratio of 20:1, the CT-26 specific IFN-γ secreting T cells in HSCT-26 DCs-immunized mice were significantly more than those in CT-26 DCs-immunized mice ($P = 0.002$). Then we evaluated the CT-26 specific cytolytic activity of splenocytes stimulated with different DC tumor vaccines. As shown in Figure 5, splenocytes from mice that received HSCT-26 DC vaccination displayed significantly stronger cytolytic activity against CT-26 cells at ratios 10:1, 20:1, and 40:1 of effector cells to target cells.

Immunization of HSCT-26 lysate-pulsed DCs protected mice with colonic cancer inoculation

We examined whether heat-shocked CT-26 cells lysate-pulsed DCs could display enhanced immunoprophylactic potential in mouse colon cancer model. After being vaccinated twice at an interval of 7 d, BALB/c mice were inoculated with CT-26 cells into cecum through open surgery. Fourteen days after the inoculation, autopsies were performed to check the growth and metastasis of colon

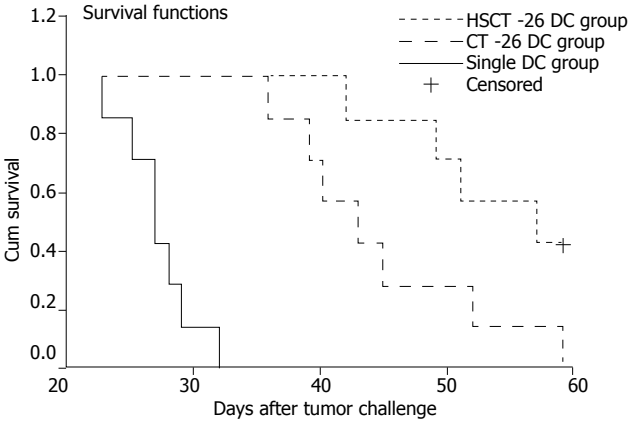

Figure 6 Survival curves of mice immunized with different dendritic cells tumor vaccine.

tumor. The mice that received CT-26 DC vaccination or single DC vaccination had colon tumor formation, whereas one of the six mice that received HSCT-26 DC vaccination was tumor free. The tumor volumes in single DC vaccination group, CT-26 DC vaccination group and HSCT-26 DC vaccination group were 107 ± 69, 24 ± 8, and 8 ± 7 mm^3, respectively. ANOVA analysis showed significant differences in tumor volume among the three groups ($P = 0.000$), but there was no difference between HSCT-26 DC vaccination group and CT-26 DC vaccination group ($P = 0.480$). Neither HSCT-26 DC vaccination group nor CT-26 DC vaccination group had peritoneal metastasis. In contrast, 50% mice in single DC vaccination group (3/6) had tumor planting to adjacent peritoneum and intestine surface accompanied with a small quantity of bloody ascites. No hepatic metastasis was found in the mice of the three groups macroscopically during autopsy.

To investigate the tumor-bearing survival time, we observed immunized mice for at least 60 d. The median survival time of mice in the single DC vaccination group, CT-26 DC vaccination group and HSCT-26 DC vaccination group was 27.0, 43.0, and 57.0 d, respectively. Log-rank test showed that the median survival time was significantly different in the three groups of mice ($P = 0.0001$). Most importantly, the mice vaccinated with HSCT-26 DCs had a much longer survival time than those vaccinated with CT-26 DCs ($P = 0.0384$). The survival curves are shown in Figure 6.

DISCUSSION

Radical surgery is the standard treatment for most patients with advanced cancer. After resection of primary tumor and local drainage lymph nodes, the tumor burden of patients is released and the immune system may have a good chance for recovery. It is the critical time to commence adjuvant immunotherapy for preventing postoperative recurrence and metastasis. The prophylactic effects of this therapy last for a long time and can be augmented by revaccination.

The anti-tumor effect of tumor cell lysate-pulsed DCs was first reported in 1998[10] and has been utilized to treat

malignant diseases including renal cancer, lymphoma, and colorectal cancer, *etc*.[11-13]. However, a misgiving of rising autoimmunity still exists as shown in animal models[14]. Current pilot clinical studies have not found clinical signs of autoimmune disease except for vitiligo and occurrence of auto antibodies. Reinhard *et al*[15] reported that vaccination with tumor lysate-pulsed DCs does not show a higher incidence of autoimmunity than vaccination with peptide-pulsed DCs. Tumor lysate is superior to other ways of DC pulsing and can elicit immunity for the entire array of TAAs of source tumors, circumventing the need of prior identification of TAAs from individual cancers. Enhancing the anti-tumor effect of whole tumor cell lysate-pulsed DCs promotes the use of this kind of tumor vaccine.

In our present study, tumor cells heated at 42 °C increased the expression of HSPs such as HSP70. Pulsed with such lysates of heat-shocked tumor cells, DCs showed higher expression of MHC-Ⅱ and co-stimulatory molecules on their surface and secreted more mIL-12 P70. As we know, DC priming T lymphocytes require signals I and II. Signal I presents the antigenic epitopes introduced by MHC molecules. Signal II as the co-stimulatory molecules on DC surface, can improve epitopes of DCs by interacting with their respective ligands on T lymphocytes. Upregulated expression of MHC-Ⅱ and co-stimulatory molecules on DC surface facilitates activation of T lymphocytes effectively. IL-12 is a cytokine leading to cell-mediated immune response (Th1 response) and is preferable for successful tumor immunotherapy[16]. Heat-induced HSPs may also serve as endogenous danger signals to drive DC maturation. Mature DCs obtain high viability and present antigenic epitopes to T lymphocytes through migrating to local drainage lymph nodes. Our result is in agreement with related reports[17]. DC activating process may be involved in NF-κB pathway[18].

It is believed that HSP-chaperoned TAAs represent the specific fingerprint of tumor cells. A gp96 receptor, CD91 on APC surface has been recently identified[19,20]. HSP-chaperoned antigen might target to APCs with the assistance of HSP receptors on these cells. HSP-peptide complexes can be internalized by APCs through receptor-mediated endocytosis[21]. HSP-mediated epitopes could go through endogenous antigen-processing pathways in the context of MHC-Ⅰ molecules[22]. DCs have an impressive ability to "cross-present" MHC class I-restricted peptide epitopes derived from exogenous proteins to CD8 T lymphocytes. In our study, compared to tumor cell lysate-pulsed DCs, immunization with heat-shocked tumor cell lysate-pulsed DCs might induce a large amount of specific CTLs in naïve mice *in vivo*. Furthermore, the induced CTLs exhibited a higher tumor specific cytotoxic activity. Immunized and challenged mouse model proved that vaccination of HSCT-26 DCs could prolong the survival time of tumor-bearing animals. All the results suggest that heating tumor cells can enhance the effect of whole tumor cell lysate-pulsed DCs to elicit specific anti-tumor response. We suppose that after taking up the HSP-chaperoned antigens, epitopes coming from HSCT-26 might be complexed with MHC class I molecules in endoplasmic reticulum and transported to the cell surface to priming CD8 T lymphocytes. Increased HSPs in tumor cells may serve as epitope chaperons and natural adjuvants in the process of DC antigen presentation, enhancing anti-tumor effects of DC vaccine.

In conclusion, heat-shocked tumor cell lysate-pulsed DCs can elicit effective anti-tumor effects *in vivo*, suppress the growth of colon cancer and eliminate occurrence of peritoneal metastasis. Using this tumor vaccine postoperatively may decrease the local recurrence and peritoneal metastasis of colon cancer and prolong the tumor-free survival time of the patients. The prophylactic effects of this DC vaccine on preventing liver metastasis of colon cancer should be further evaluated.

REFERENCES

1 **Banchereau J**, Schuler-Thurner B, Palucka AK, Schuler G. Dendritic cells as vectors for therapy. *Cell* 2001; **106**: 271-274
2 **Whiteside TL**, Odoux C. Dendritic cell biology and cancer therapy. *Cancer Immunol Immunother* 2004; **53**: 240-248
3 **Schuler G**, Schuler-Thurner B, Steinman RM. The use of dendritic cells in cancer immunotherapy. *Curr Opin Immunol* 2003; **15**: 138-147
4 **Mocellin S**, Rossi CR, Lise M, Nitti D. Colorectal cancer vaccines: principles, results, and perspectives. *Gastroenterology* 2004; **127**: 1821-1837
5 **Audibert F**. Adjuvants for vaccines, a quest. *Int Immunopharmacol* 2003; **3**: 1187-1193
6 **Ménoret A**, Patry Y, Burg C, Le Pendu J. Co-segregation of tumor immunogenicity with expression of inducible but not constitutive hsp70 in rat colon carcinomas. *J Immunol* 1995; **155**: 740-747
7 **Okamoto M**, Tazawa K, Kawagoshi T, Maeda M, Honda T, Sakamoto T, Tsukada K. The combined effect against colon-26 cells of heat treatment and immunization with heat treated colon-26 tumour cell extract. *Int J Hyperthermia* 2000; **16**: 263-273
8 **Lutz MB**, Kukutsch N, Ogilvie AL, Rössner S, Koch F, Romani N, Schuler G. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J Immunol Methods* 1999; **223**: 77-92
9 **Miyahira Y**, Murata K, Rodriguez D, Rodriguez JR, Esteban M, Rodrigues MM, Zavala F. Quantification of antigen specific CD8+ T cells using an ELISPOT assay. *J Immunol Methods* 1995; **181**: 45-54
10 **Fields RC**, Shimizu K, Mulé JJ. Murine dendritic cells pulsed with whole tumor lysates mediate potent antitumor immune responses in vitro and in vivo. *Proc Natl Acad Sci U S A* 1998; **95**: 9482-9487
11 **Höltl L**, Zelle-Rieser C, Gander H, Papesh C, Ramoner R, Bartsch G, Rogatsch H, Barsoum AL, Coggin JH, Thurnher M. Immunotherapy of metastatic renal cell carcinoma with tumor lysate-pulsed autologous dendritic cells. *Clin Cancer Res* 2002; **8**: 3369-3376
12 **Maier T**, Tun-Kyi A, Tassis A, Jungius KP, Burg G, Dummer R, Nestle FO. Vaccination of patients with cutaneous T-cell lymphoma using intranodal injection of autologous tumor-lysate-pulsed dendritic cells. *Blood* 2003; **102**: 2338-2344
13 **Rains N**, Cannan RJ, Chen W, Stubbs RS. Development of a dendritic cell (DC)-based vaccine for patients with advanced colorectal cancer. *Hepatogastroenterology* 2001; **48**: 347-351
14 **Ludewig B**, Ochsenbein AF, Odermatt B, Paulin D, Hengartner H, Zinkernagel RM. Immunotherapy with dendritic cells directed against tumor antigens shared with normal host cells results in severe autoimmune disease. *J Exp Med* 2000; **191**: 795-804
15 **Reinhard G**, Märten A, Kiske SM, Feil F, Bieber T, Schmidt-Wolf IG. Generation of dendritic cell-based vaccines for cancer therapy. *Br J Cancer* 2002; **86**: 1529-1533
16 **Mashino K**, Sadanaga N, Tanaka F, Ohta M, Yamaguchi H, Mori M. Effective strategy of dendritic cell-based

immunotherapy for advanced tumor-bearing hosts: the critical role of Th1-dominant immunity. *Mol Cancer Ther* 2002; **1**: 785-794

17 **Kuppner MC**, Gastpar R, Gelwer S, Nössner E, Ochmann O, Scharner A, Issels RD. The role of heat shock protein (hsp70) in dendritic cell maturation: hsp70 induces the maturation of immature dendritic cells but reduces DC differentiation from monocyte precursors. *Eur J Immunol* 2001; **31**: 1602-1609

18 **Basu S**, Binder RJ, Suto R, Anderson KM, Srivastava PK. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. *Int Immunol* 2000; **12**: 1539-1546

19 **Wassenberg JJ**, Dezfulian C, Nicchitta CV. Receptor mediated and fluid phase pathways for internalization of the ER Hsp90 chaperone GRP94 in murine macrophages. *J Cell Sci* 1999; **112 (Pt 13)**: 2167-2175

20 **Binder RJ**, Han DK, Srivastava PK. CD91: a receptor for heat shock protein gp96. *Nat Immunol* 2000; **1**: 151-155

21 **Srivastava PK**, Amato RJ. Heat shock proteins: the 'Swiss Army Knife' vaccines against cancers and infectious agents. *Vaccine* 2001; **19**: 2590-2597 22 **Singh-Jasuja H**, Hilf N, Arnold-Schild D, Schild H. The role of heat shock proteins and their receptors in the activation of the immune system. *Biol Chem* 2001; **382**: 629-636

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RAPID COMMUNICATION

Intestinal permeability in rats with CCl_4-induced portal hypertension

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Abstract

AIM: To investigate the intestinal barrier changes in rats with CCl_4-induced portal hypertension.

METHODS: The permeability of intestinal barrier detected by Lanthanum as a tracer was evaluated in rats. Bacterial translocation and plasma endotoxin were also determined.

RESULTS: The incidence of bacterial translocation was 85% in rats with CCl_4-induced portal hypertension, which was significantly higher than that in control rats (20%, $P<0.01$). Plasma endotoxin level was significantly higher in experimental group than in control group. Permeability of the epithelial mucosa and pathological alteration were increased in the ileum and the microvilli became shorter and thinner in rats with portal hypertension.

CONCLUSION: Bacterial translocation occurs in rats with CCl_4-induced portal hypertension and increased permeability between epithelial cells contributes to the translocation.

Key words: Portal hypertension; Bacterial translocation; Intestinal barrier

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INTRODUCTION

The incidence of bacterial infection in patients with portal hypertension is higher than that in the general population[1]. Most of these infections are caused by bacteria normally present in the intestine. Portal hypertension may play an important role in the pathogenesis of infections in cirrhotic patients[2]. On the other hand, endotoxemia present in patients with portal hypertension is correlated with the severity of the disease. Recently, bacterial translocation from the gut to extraintestinal organs and systemic circulation has been proved in patients with other diseases[3] and is considered as the cause of endotoxemia in patients due to the disruption of the gut barrier function. However, the exact route of bacterial translocation and endotoxemia in patients with portal hypertension is not clear. In the present study, bacterial translocation, permeability of the enterocyte membrane and intestinal morphological alterations in rats with CCl_4-induced portal hypertension were evaluated by transmission electroscopy.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats ($n=40$) weighing 250-300 g were used. The animals were allowed to acclimate to laboratory condition for 4-6 d with free access to water and rat chow before the experiment.

Twenty rats entered the experimental group and received intragastric CCl_4 via a Hamilton syringe with an attached stainless steel animal feeding tube after light ether anesthesia. The first dose of CCl_4 was 20 μL and subsequent doses were adjusted based on the body weight 48 h after the last dose. After the appearance of ascites, the dose was reduced to 40 μL and increased according to the schedule if ascites resolved. The other 20 rats were gavaged with water and served as control group.

Studies on intestinal permeability and bacterial translocation from the gut were carried out on d 14. The abdominal cavity was opened by a midline incision and blood samples were immediately inoculated into 3 mL blood culture medium and 0.5 mL plasma was used to detect endotoxin. Limulus-amoebocyte-lysate (LAL) test was used to determine plasma endotoxin levels as previously described[4]. *Escherichia coli* 055 was used as endotoxin standard. The results were expressed as endotoxin units per milliliter (EU/mL). The mesenteric lymph nodes (MLNs) near the distal ileum, liver, and spleen were harvested and weighed, tissues were then homogenized in a test tube containing 3 mL brain heart infusion broth, 0.2 mL supernatant was taken for bacterial culture. All media for aerobic culture were incubated at 37 °C for

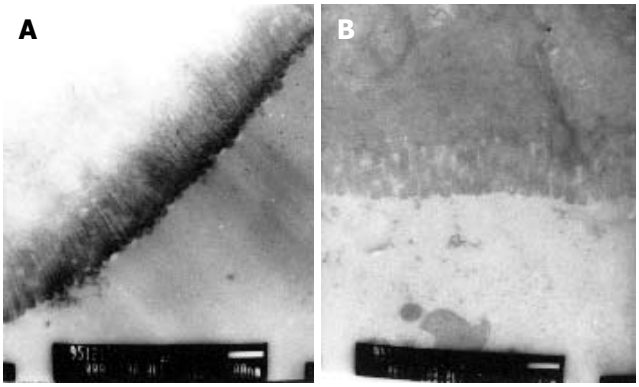

Figure 1 Normal microvilli of enterocytes in control rats (**A**) and shortened and thinned microvilli of enterocytes in rats with portal hypertension (**B**).

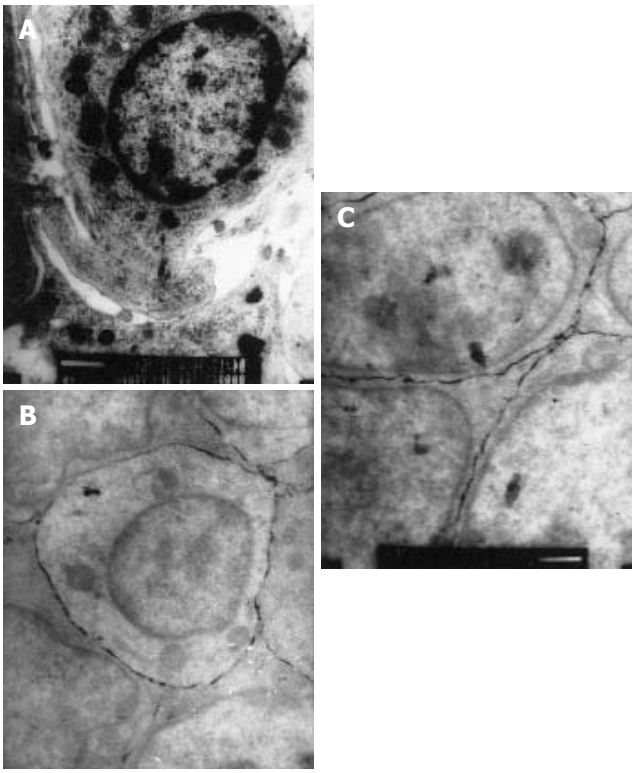

Figure 2 Lanthanum tracer in the ileum of control rats (**A**) and rats with CCl_4-induced portal hypertension (**B** and **C**).

3-5 d and isolated bacteria were identified by standard procedures. At the end of the experiment, a segment of the distal ileum was removed and placed into 2.5% glutaraldehyde fixative with 4% lanthanum hydroxide for 1 h, then washed thrice to assay the permeability of the intestinal barrier. Sections were made from each sample and the presence of the tracer was observed under transmission electron microscope.

Positive MLN culture was considered indicative of bacterial translocation from the gut. Positive blood, spleen or liver cultures were considered indicative of passage bacteria to the systemic circulation.

Statistical analysis

Translocation incidence was analyzed by the Fisher's exact test. Weights and other data were expressed as mean ± SD and compared with Student's *t* test.

RESULTS

The mean spleen weight in rats with CCl_4-induced portal hypertension (5.32 ± 0.38 mg/g body wt) was significantly greater than that in control rats (2.31 ± 0.28 mg/g body wt). The weight of MLN was also greater in rats with CCl_4-induced portal hypertension (1.85 ± 0.42 mg/g body wt) than that in control rats (0.86 ± 0.25 mg/g body wt). Ascites occurred in all rats.

Bacterial translocation to MLN occurred in 17 of 20 (85%) CCl_4-PH rats and in 4 of 20 (20%) control rats. Bacteria were found in ascites in the experimental group (7/20, 35%) while no bacteria were found in the control group. All bacteria isolated from MLNs and ascites were Gram-negative. No bacteria were isolated from blood, spleen or liver.

Plasma levels of endotoxin (0.083±0.012 EU/mL) and ascites (0.062 ± 0.012 EU/mL) were significantly higher in the experimental group than in the control group (0.046 ± 0.009 EU/mL, $P < 0.01$).

The distal ileum of rats with CCl_4-induced portal hypertension did not differ grossly from that of the control rats. Clear and intact microvilli were seen in ileal enterocytes in the control rats. Shortened and thinned microvilli appeared in rats with CCl_4-induced portal hypertension (Figure 1).

The tracer did not penetrate into the cells or intercellular juncture in the control rats. The tracer appeared between enterocytes and cellular junctions in rats with CCl_4-induced portal hypertension. The penetrated tracer aggregated along the cellular junctions (tight or gap) or within the mucosal propria lamina without entering enterocytes or winding lines (Figure 2).

DISCUSSION

The mechanism underlying bacterial penetration into the intestinal barrier and entry into the systemic circulation in cirrhotic patients remains unclear[5-7]. Recent studies in animal model demonstrated that bacteria from the gastrointestinal tract can cross over the intestinal barrier to infect extraintestinal sites and/or the systemic circulation, a process known as bacterial translocation[8-10]. Various pathological changes such as damage of the intestinal barrier can increase the incidence of bacterial translocation[11]. Cirrhotic patients usually develop portal hypertension that causes intestinal congestion, thus inducing intestinal mucosal abnormalities[12], suggesting that portal hypertension may play a prominent role in the pathogenesis of spontaneous bacteremia and peritonitis in cirrhotic patients. Our results indicated that bacterial translocation occurred in 85% portal hypertensive rats, which was significantly higher (20%) than that in the control rats. Surgical manipulation and stress may be responsible for the translocation of bacteria[13-15].

The reason why permeability of the intestinal mucosa is increased remains unclear. In the present study, the tracer (Lanthanum) with a mean diameter of 4 nm is normally located outside the cell membrane, but may

enter the cells through cellular pores, while their diameter exceeds 2 nm. Our results showed that the permeability of intestinal barrier was greatly increased in rats with portal hypertension. The tracer appeared between enterocytes and lamina propria cells, indicating that translocation occurs between enterocytes and lamina propria cells. This finding is consistent with the observation of Cole *et al*[17]. The damaged microvilli in rats with CCl_4-induced portal hypertension demonstrated that permeability changes might occur before the development of pathological abnormality[15-17].

In conclusion, bacterial translocation occurs in rats with CCl_4-induced portal hypertension and the increased permeability between epithelial cells contributes to the translocation.

REFERENCES

1 **Samonakis DN**, Triantos CK, Thalheimer U, Patch DW, Burroughs AK. Management of portal hypertension. *Postgrad Med J* 2004; **80**: 634-641
2 **Wiest R**, Garcia-Tsao G. Bacterial translocation (BT) in cirrhosis. *Hepatology* 2005; **41**: 422-433
3 **Garcia-Tsao G**, Wiest R. Gut microflora in the pathogenesis of the complications of cirrhosis. *Best Pract Res Clin Gastroenterol* 2004; **18**: 353-372
4 **Hashimoto N**, Ohyanagi H. Effect of acute portal hypertension on gut mucosa. *Hepatogastroenterology* 2002; **49**: 1567-1570
5 **Chiva M**, Guarner C, Peralta C, Llovet T, Gómez G, Soriano G, Balanzó J. Intestinal mucosal oxidative damage and bacterial translocation in cirrhotic rats. *Eur J Gastroenterol Hepatol* 2003; **15**: 145-150
6 **Solà R**, Soriano G. Why do bacteria reach ascitic fluid? *Eur J Gastroenterol Hepatol* 2002; **14**: 351-354
7 **Cirera I**, Bauer TM, Navasa M, Vila J, Grande L, Taurá P, Fuster J, García-Valdecasas JC, Lacy A, Suárez MJ, Rimola A, Rodés J. Bacterial translocation of enteric organisms in patients with cirrhosis. *J Hepatol* 2001; **34**: 32-37
8 **Caly WR**, Strauss E. A prospective study of bacterial infections in patients with cirrhosis. *J Hepatol* 1993; **18**: 353-358
9 **Sheer TA**, Runyon BA. Spontaneous bacterial peritonitis. *Dig Dis* 2005; **23**: 39-46
10 **Ramachandran A**, Prabhu R, Thomas S, Reddy JB, Pulimood A, Balasubramanian KA. Intestinal mucosal alterations in experimental cirrhosis in the rat: role of oxygen free radicals. *Hepatology* 2002; **35**: 622-629
11 **Deitch EA**. Simple intestinal obstruction causes bacterial translocation in man. *Arch Surg* 1989; **124**: 699-701
12 **Groszmann RJ**, Abraldes JG. Portal hypertension: from bedside to bench. *J Clin Gastroenterol* 2005; **39**: S125-S130
13 **Lora L**, Mazzon E, Martines D, Fries W, Muraca M, Martin A, d'Odorico A, Naccarato R, Citi S. Hepatocyte tight-junctional permeability is increased in rat experimental colitis. *Gastroenterology* 1997; **113**: 1347-1354
14 **Runyon BA**, Borzio M, Young S, Squier SU, Guarner C, Runyon MA. Effect of selective bowel decontamination with norfloxacin on spontaneous bacterial peritonitis, translocation, and survival in an animal model of cirrhosis. *Hepatology* 1995; **21**: 1719-1724
15 **Alexander JW**, Boyce ST, Babcock GF, Gianotti L, Peck MD, Dunn DL, Pyles T, Childress CP, Ash SK. The process of microbial translocation. *Ann Surg* 1990; **212**: 496-510; discussion 511-2
16 **Deitch EA**, Bridges RM. Effect of stress and trauma on bacterial translocation from the gut. *J Surg Res* 1987; **42**: 536-542
17 **Cole GT**, Seshan KR, Pope LM, Yancey RJ. Morphological aspects of gastrointestinal tract invasion by Candida albicans in the infant mouse. *J Med Vet Mycol* 1988; **26**: 173-185

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RAPID COMMUNICATION

Serum soluble interleukin-2 receptor levels in patients with chronic hepatitis B virus infection and its relation with anti-HBc

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Abstract

AIM: To investigate the relationship between serum soluble interleukin-2 receptor (sIL-2R) level and anti-HBc in patients with chronic hepatitis B virus (HBV) infection.

METHODS: Sera from 100 patients with chronic HBV infection and 30 healthy controls were included in this study. The patients were divided into group A [HBsAg (+), HBeAg (+) and anti-HBc (+), $n = 50$] and group B [HBsAg (+), HBeAg (+) and anti-HBc (−), $n = 50$]. sIL-2R levels were determined using ELISA. HBV DNA and alanine aminotransferase (ALT) were also detected.

RESULTS: Serum sIL-2R levels were significantly higher in patients with chronic HBV infection than in healthy controls. Moreover, serum sIL-2R levels were significantly higher in patients with HBsAg (+), HBeAg (+) and anti-HBc (+) ($976.56 \pm 213.51 \times 10^3$ U/L) than in patients with HBsAg (+), HBeAg (+) and anti-HBc (−) ($393.41 \pm 189.54 \times 10^3$ U/L, $P < 0.01$). A significant relationship was found between serum sIL-2R and ALT levels ($P < 0.01$) in patients with chronic HBV infection, but there was no correlation between sIL-2R and HBV DNA levels. The anti-HBc status was significantly related to the age of patients ($P < 0.01$).

CONCLUSION: The high sIL-2R level is related to positive anti-HBc in chronic hepatitis B patients. Positive anti-HBc may be related to T-lymphocyte activation and negative anti-HBc may imply immune tolerance in these patients.

Key words: Chronic hepatitis B; Hepatitis B virus; Anti-HBc; Soluble interleukin-2 receptor; Immune tolerance

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INTRODUCTION

About 350 million persons are chronically infected with hepatitis B virus (HBV) in the world[1]. Carriers of HBV are at an increased risk of developing cirrhosis, hepatic decompensation and hepatocellular carcinoma (HCC)[2]. China has the greatest burden of hepatitis B and liver cancer in the world. A third of all chronic HBV carriers live in China. Each year, about half a million Chinese die of liver cancer or liver failure due to hepatitis B. However, HBV has no cytopathic effect on hepatocytes. Some liver damages caused by HBV are attributed to immune clearance of virus-infected cells and associated immune reactions. While antibody response in patients with HBV infection plays a critical role in viral clearance through the formation of complexes with viral particles and their removal from the circulation[3], specific cellular immune response plays a main role in hepatic necrosis due to HBV infection and in the persistence of viral infection[4].

IL-2R system plays an important role in the activation and proliferation of lymphocytes[5]. IL-2R is expressed on the cell membrane of lymphocytes and contains at least three different chains. Serum sIL-2R is predominantly released from activated T lymphocytes and can serve as an index of activation of T lymphocytes[6]. Serum sIL-2R levels are significantly higher in patients with chronic HBV infection than in healthy controls[7]. The serum sIL-2R level one year after interferon administration may be a useful marker of interferon's therapeutic effectiveness[8].

In the present study, we determined the serum levels of sIL-2R in chronic hepatitis B patients with positive or negative anti-HBc to analyze the elevated patterns of sIL-2R in patients with different anti-HBc status.

MATERIALS AND METHODS

Patients

Serum samples were obtained from 100 Chinese patients

Table 1 Levels of sIL-2R, ALT, and HBV DNA in sera of patients with chronic HBV infection (mean ± SD)

Group	Number	sIL-2R ($\times 10^3$ U/L)	ALT (IU/L)	HBV DNA (copies/mL, lgC)
A	50	967.56±213.51[b]	79±21.2[d]	7.954±1.754
B	50	393.41±189.54[a]	24±12.3	7.875±1.011
Control	30	243.59±121.34	13±6.5	0

[b]$P<0.01$ *vs* group B and control group; [a]$P<0.05$ *vs* control group; [d]$P<0.01$ *vs* group B and control.

positive for HBsAg and HBeAg. All the patients were followed up from 2001 to 2004. The patients were divided into two groups. Group A consisted of 50 patients positive for HBsAg, HBeAg, and anti-HBc (age range, 3-71 years; mean age, 37 years). Group B consisted of 50 patients positive for HBsAg and HBeAg but negative for anti-HBc (age range, 12-33 years; mean age, 22.5 years). Another 30 healthy persons negative for all HBV markers served as the control group.

Blood sampling

Venous blood samples were taken to detect sIL-2R, alanine aminotransferase (ALT), HBV DNA and HBV markers (HBsAg, HBeAg, and anti-HBc). All serum samples were separated and stored at -20 °C until testing.

Determination of sIL-2R

Serum sIL-2R levels were determined by sandwich ELISA using commercially available sIL-2R assay kits (Department of Immunology, Dr Bethune Medical University, Changchun, China). An anti-sIL2R monoclonal antibody was adsorbed onto the substrate of polystyrene microtiter wells. The sIL-2R present in the samples or in the standard solutions was bound to the antibody-coated wells. The unbound sample components were removed by washing thrice. A peroxidase-linked anti-sIL-2R monoclonal antibody against another epitope on the sIL-2R molecule was then added to complete the sandwich. After being washed, the unbound materials were removed and a substrate solution was added into the wells. A stopping solution was added to stop the reaction and then light absorbance at 492 nm was measured. A standard curve was prepared from four IL-2R standards. The values were expressed as unit (U) per L.

Assay of HBV markers and HBV DNA

HBsAg, HBeAg, and anti-HBc were detected using commercially available EIA or ELISA kits (Reagents Development Center, Shanghai Hospital for Infectious Diseases, Shanghai, China). HBV DNA levels were tested using real-time PCR on ABI 7000 real-time detection system (Applied Biosystems, Foster City, CA, USA).

Measurement of ALT

ALT was tested on a CX4 chemistry analyzer (Beckman Coulter, Fullerton, CA, USA) using commercially available kits.

Table 2 Age difference between anti-HBc (+) and anti-HBc (–) patients with chronic HBV infection

Group	Number	Median (yr)	Rank sum
A	50	37[b]	66.88
B	50	22.5	40.12

[b]$P<0.0001$ *vs* group B.

Statistical analysis

The significance of difference between the two groups was determined with Student's *t* test and Wilcoxon's rank-sum test. $P<0.05$ was considered significant.

RESULTS

Serum sIL-2R levels were significantly higher in patients with chronic HBV infection than in healthy controls. Moreover, serum sIL-2R levels were significantly higher in patients with HBsAg (+), HBeAg (+) and anti-HBc (+) (group A, $976.56 \pm 213.51 \times 10^3$ U/L) than in patients with HBsAg (+), HBeAg (+) and anti-HBc (–) (group B, $393.41 \pm 189.54 \times 10^3$ U/L, $P< 0.01$). ALT levels were significantly higher in group A ($79 \pm 21.2 \times 10^3$ U/L) than in group B ($24 \pm 12.3 \times 10^3$ U/L, $P<0.01$). Serum sIL-2R levels were significantly related to ALT levels. There was no significant difference in HBV DNA levels between the two groups (Table 1). Anti-HBc status was related to the age of the patients. Positive anti-HBc was detected in older patients ($P< 0.01$, Table 2).

DISCUSSION

HBV infection is a major health problem. About 350 million persons are chronically infected with HBV in the world. HBV itself is non-cytopathic and it is widely accepted that the mechanism of hepatocellular injury is the host anti-viral immune response[9]. A human leukocyte antigen (HLA) class I-restricted cytotoxic T-lymphocyte (CTL) response to one or more HBV-encoded antigens on the hepatocyte membrane is a major mechanism of hepatocellular injury and clearance of infected cells[10]. Serum sIL-2R is predominantly released from activated T lymphocytes[11]. It was reported that serum sIL-2R levels reflect cellular IL-2 receptor expression[6]. Hence, levels of serum sIL-2R are useful in monitoring T-lymphocyte activity and serial measurement aids in assessing the progression of the disease[12].

High levels of serum sIL-2R have been observed in patients with chronic HBV infection[7,8,12-14] and hepatitis C virus (HCV) infection[15]. Serum sIL-2R levels indicate the degree of liver damage in patients with chronic HBV infection[8]. Our results showed that serum sIL-2R levels were significantly higher in patients with chronic HBV infection than in healthy controls. The serum sIL-2R levels were significantly related to the serum ALT levels, but did not correlate with serum HBV DNA levels in patients with chronic HBV infection. These results are consistent with previous findings of Sawayama *et al*[8].

Anti-HBc is detected in virtually all patients exposed

to HBV[16] and typically persists for life[17]. However, many patients with chronic HBV infection are negative for anti-HBc in China probably due to the fact that Chinese people acquire the infection at birth or during the early postnatal period[18,19]. These patients may have an immune tolerance to the virus for several decades of life[20,21]. We found that serum sIL-2R levels were significantly higher in patients with HBsAg (+), HBeAg (+) and anti-HBc (+) than in patients with HBsAg (+), HBeAg (+) and anti-HBc (–). Furthermore, patients with anti-HBc (+) were older than those with anti-HBc (–). Transfer of hepatitis B core antigen-reactive T cells is associated with the resolution of chronic HBV infection[22]. Based on these results, it seems that patients with chronic HBV infection who are negative for anti-HBc may be in a status of immune tolerance to the virus. Serum sIL-2R levels and anti-HBc may be useful indicators of immune status in patients with chronic HBV infection. Serum sIL-2R levels reflect the activation of T lymphocytes[5]. Hence, positive anti-HBc may be related to the activation of T lymphocytes.

Though interferon alpha to some extent hastens the loss of HBeAg in Chinese patients, the treatment is generally less effective than in white patients. This is probably due to the fact that the majority of Chinese people have a long period of immune tolerance to the virus[23]. Several studies have revealed that serum sIL-2R levels can serve as an index of the activation of T lymphocytes[5,6]. The serum sIL-2R level one year after interferon administration may be a useful marker of its therapeutic effectiveness[8]. Our results showed that elevated serum sIL-2R and positive anti-HBc were related to the high levels of serum ALT in patients with chronic HBV infection. Low serum ALT levels are associated with the poor response to interferon alpha treatment in patients with chronic HBV infection[23]. Hence, we can deduce that elevated serum sIL-2R levels, positive anti-HBc and high ALT concentrations may serve as indicators for interferon alpha treatment in Chinese patients with chronic HBV infection. However, further exploration is needed.

In conclusion, serum sIL-2R levels are related to anti-HBc and serum ALT concentrations, but not related to HBV DNA levels in patients with chronic HBV infection. Positive anti-HBc may be related to T-lymphocyte activation and negative anti-HBc may imply immune tolerance in these patients.

REFERENCES

1 **Lee WM**. Hepatitis B virus infection. *N Engl J Med* 1997; **337**: 1733-1745
2 **Beasley RP**. Hepatitis B virus. The major etiology of hepatocellular carcinoma. *Cancer* 1988; **61**: 1942-1956
3 **Böcher WO**, Galun E, Marcus H, Daudi N, Terkieltaub D, Shouval D, Löhr HF, Reisner Y. Reduced hepatitis B virus surface antigen-specific Th1 helper cell frequency of chronic HBV carriers is associated with a failure to produce antigen-specific antibodies in the trimera mouse. *Hepatology* 2000; **31**: 480-487
4 **Monsalve-De Castillo F**, Romero TA, Estévez J, Costa LL, Atencio R, Porto L, Callejas D. Concentrations of cytokines, soluble interleukin-2 receptor, and soluble CD30 in sera of patients with hepatitis B virus infection during acute and convalescent phases. *Clin Diagn Lab Immunol* 2002; **9**: 1372-1375
5 **Lai KN**, Leung JC, Tam JS, Leung NW. T lymphocyte activation in chronic hepatitis B infection: interleukin 2 release and its receptor expression. *Am J Gastroenterol* 1989; **84**: 1532-1537
6 **Rubin LA**, Nelson DL. The soluble interleukin-2 receptor: biology, function, and clinical application. *Ann Intern Med* 1990; **113**: 619-627
7 **Müller C**, Knoflach P, Zielinski CC. Soluble interleukin 2 receptor in acute viral hepatitis and chronic liver disease. *Hepatology* 1989; **10**: 928-932
8 **Sawayama Y**, Hayashi J, Kawakami Y, Furusyo N, Ariyama I, Kishihara Y, Ueno K, Kashiwagi S. Serum soluble interleukin-2 receptor levels before and during interferon treatment in patients with chronic hepatitis B virus infection. *Dig Dis Sci* 1999; **44**: 163-169
9 **Ilan Y**. Immune downregulation leads to upregulation of an antiviral response: a lesson from the hepatitis B virus. *Microbes Infect* 2002; **4**: 1317-1326
10 **Franzese O**, Kennedy PT, Gehring AJ, Gotto J, Williams R, Maini MK, Bertoletti A. Modulation of the CD8+-T-cell response by CD4+ CD25+ regulatory T cells in patients with hepatitis B virus infection. *J Virol* 2005; **79**: 3322-3328
11 **Rubin LA**, Galli F, Greene WC, Nelson DL, Jay G. The molecular basis for the generation of the human soluble interleukin 2 receptor. *Cytokine* 1990; **2**: 330-336
12 **Leung NW**, Leung JC, Tam JS, Lau JT, Lai KN. Effects of alpha-interferon and prednisone on serum-soluble interleukin-2 receptor (sIL-2R) in chronic hepatitis B infection. *Am J Gastroenterol* 1992; **87**: 113-117
13 **Alberti A**, Chemello L, Fattovich G, Pontisso P, Semenzato G, Colletta C, Vinante F, Pizzolo G. Serum levels of soluble interleukin-2 receptors in acute and chronic viral hepatitis. *Dig Dis Sci* 1989; **34**: 1559-1563
14 **Casafont F**, Echevarria S, Pons Romera F. Interleukin-2 activity and serum levels of soluble interleukin-2 receptors in chronic active hepatitis B. *Dig Dis Sci* 1990; **35**: 1045
15 **Hayashi J**, Kishihara Y, Yamaji K, Yoshimura E, Ohmiya M, Tani Y, Ikematsu H, Kashiwagi S. Serum levels of soluble interleukin-2 receptors and effects of interferon-alpha for patients with chronic hepatitis C virus. *Dig Dis Sci* 1995; **40**: 1837-1841
16 **Jung MC**, Pape GR. Immunology of hepatitis B infection. *Lancet Infect Dis* 2002; **2**: 43-50
17 **Seeff LB**, Beebe GW, Hoofnagle JH, Norman JE, Buskell-Bales Z, Waggoner JG, Kaplowitz N, Koff RS, Petrini JL, Schiff ER. A serologic follow-up of the 1942 epidemic of post-vaccination hepatitis in the United States Army. *N Engl J Med* 1987; **316**: 965-970
18 **Yuen MF**, Hui CK, Cheng CC, Wu CH, Lai YP, Lai CL. Long-term follow-up of interferon alfa treatment in Chinese patients with chronic hepatitis B infection: The effect on hepatitis B e antigen seroconversion and the development of cirrhosis-related complications. *Hepatology* 2001; **34**: 139-145
19 **Lai CL**, Lin HJ, Lau JN, Flok AS, Wu PC, Chung HT, Wong LK, Leung MP, Yeung CY. Effect of recombinant alpha 2 interferon with or without prednisone in Chinese HBsAg carrier children. *Q J Med* 1991; **78**: 155-163
20 **Lok AS**, Lai CL, Wu PC, Lau JY, Leung EK, Wong LS, Fung YL. Alpha-interferon treatment in Chinese patients with chronic hepatitis B. *J Hepatol* 1990; **11 Suppl 1**: S121-S125
21 **Derso A**, Boxall EH, Tarlow MJ, Flewett TH. Transmission of HBsAg from mother to infant in four ethnic groups. *Br Med J* 1978; **1**: 949-952
22 **Lau GK**, Suri D, Liang R, Rigopoulou EI, Thomas MG, Mullerova I, Nanji A, Yuen ST, Williams R, Naoumov NV. Resolution of chronic hepatitis B and anti-HBs seroconversion in humans by adoptive transfer of immunity to hepatitis B core antigen. *Gastroenterology* 2002; **122**: 614-624
23 **Yuen MF**, Lai CL. Treatment of chronic hepatitis B. *Lancet Infect Dis* 2001; **1**: 232-241

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RAPID COMMUNICATION

Role of nitric oxide in Toll-like receptor 2 and 4 mRNA expression in liver of acute hemorrhagic necrotizing pancreatitis rats

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Abstract

AIM: To investigate the role of nitric oxide (NO) in Toll-like receptor 2 (TLR2)/4mRNA expression in livers of acute hemorrhagic necrotizing pancreatitis (AHNP) rats.

METHODS: One hundred and ten SD male rats were randomly divided into sham-operated group ($n = 10$), AHNP group ($n = 30$), chloroquine (CQ)-treated group ($n = 30$) and L-Arg-treated group ($n = 40$). TLR2/4mRNA expression in the liver of AHNP rats was measured by RT-PCR.

RESULTS: Expression of TLR2/4mRNA could be detected in the liver of AHNP rats in sham-operated group (0.155E-5 ± 0.230E-6 and 0.115E-2 ± 0.545E-4), but was markedly increased at 3 h in AHNP group (0.197E-2 ± 0.114E-3 and 0.175 ± 0.349E-2) peaking at 12 h (0.294E-2 ± 0.998E-4 and 2.673 ± 2.795E-2, $P < 0.01$). Hepatic injuries were aggravated, TNF-α concentration in the liver was increased and NO concentration was decreased ($P < 0.05$ or $P < 0.01$). When TLR2/4mRNA expression was inhibited by CQ (3 h: 1.037E-4 ± 3.299E-6 and 0.026 ± 3.462E-3; 6 h: 1.884E-4 ± 4.679E-6 and 0.108 ± 6.115E-3; 12 h: 2.443E-4 ± 7.714E-6 and 0.348 ± 6.807E-3; $P < 0.01$), hepatic injuries were relieved, NO concentration in the liver was increased and TNF-α concentration was decreased ($P < 0.05$ or $P < 0.01$). When rats with AHNP were treated with L-Arg, TLR2/4mRNA expression in the liver could be effectively inhibited (50 mg-T: 0.232E-2 ± 0.532E-4 and 0.230 ± 6.883E-3; 100 mg-T: 0.210E-2 ± 1.691E-4 and 0.187 ± 0.849E-2; 200 mg-T: 0.163E-2 ± 0.404E-4 and 0.107 ± 0.195E-2; 400 mg-T: 0.100E-2 ± 0.317E-4 and 0.084 ± 0.552E-2; $P < 0.01$) and hepatic injuries were relieved. At the same time, NO concentration in the liver was markedly increased and TNF-α concentration was decreased ($P < 0.05$ or $P < 0.01$).

CONCLUSION: The expression of TLR2/4mRNA is increased and hepatic injuries are aggravated in the liver of AHNP rats. TLR2/4mRNA gene expression in the liver of AHNP rats can be markedly inhibited by NO, leading to the relief of hepatic injuries.

Key words: Toll-like receptors; Acute hemorrhage necrotizing pancreatitis; Liver; Nitric oxide; Chloroquine

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INTRODUCTION

Acute hemorrhagic necrotizing pancreatitis (AHNP) is a serious disease of human beings with a high mortality and morbidity. AHNP could cause multiple organ dysfunction syndrome (MODS). Unfortunately, the pathogenesis and mechanism of AHNP are still unclear. Many researches indicate that diverse inflammatory factors such as tumor necrosis factor (TNF-α), interleukin-1 (IL-1), IL-6 and reactive oxygen species result in systemic inflammatory response syndrome (SIRS) which might play an important role in the pathogenesis and development of AHNP[1-3]. It was reported that Toll-like receptor 2 (TLR2)/4 activated by stimulations can result in excessive production and release of cytokines[4]. In the present study, we have investigated the changes of TLR2/4 gene expression and the effect of NO on TLR2/4 gene expression in livers of AHNP rats.

Table 1 Serum amylase, ALT, and AST concentrations (mean±SD)

		n	Serum amylase (U/L)	ALT (U/L)	AST (U/L)
A	unit	10	985±159.68	74.0±4.47	176.6±4.52
B	unit	10	6 367±1 122.17[b]	101.8±4.11[a]	447.9±54.49[b]
C	unit	10	9 370±2 282.79[b]	232.9±24.01[b]	1055.9±41.57[b]
D	unit	10	13 189±3 365.14[b]	546.5±37.36[b]	1276.2±44.22[b]
E	unit	10	3 450±711.25[bd]	95.5±4.19[b]	370.3±19.67[b]
F	unit	10	4 165±1 005.31[b,c,e]	119.7±4.74[b,d]	784.6±68.93[b,d]
G	unit	10	5 540±1 274.81[b,c,e]	197.5±9.09[b,d]	982.7±46.22[b,d]
H	unit	10	6 793±1 414.78[b]	212.8±5.50[b]	854.3±53.26[b,d]
I	unit	10	6 518±246.13[b,c]	142.2±7.73[b,d]	405.9±62.62[b,d]
J	unit	10	5 462±822.44[b,c]	115.4±6.30[b,d]	385.4±6.72[b,c,d]
K	unit	10	4 789±826.59[b,c]	92.3±3.69[b,d]	309.7±15.90[b,d]

[a]$P<0.05$, [b]$P<0.01$ *vs* A unit; [c]$P>0.05$ *vs* ahead unit; [e]$P<0.05$, [d]$P<0.01$ *vs* unit at the same time point of AHNP group.

MATERIALS AND METHODS

Chloroquine (CQ) and sodium taurocholate (TAC) were purchased from Sigma (St. Louis, MO, USA). L-Arg was purchased from Cayman Chemical Company, USA. Trizol was purchased from Promega Co., Hong Kong, China. Reverse transcriptase RNase and DNA polymerase were purchased from TOYOBO CO., LTD, Japan. Serum-amylase and NO detection kits were provided by Jiancheng Biological Engineering Research Institute, Nanjing, China.

Groups and models

One hundred and ten SD male rats (weighing 180-200 g) were purchased from Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. Rats were randomized into sham-operated group ($n=10$), AHNP group (3, 6, and 12 h, units B-D, $n=10$), CQ-treated group (3, 6, and 12 h, units E-G, $n=10$), L-Arg-treated group (50, 100, 200, and 400 mg; units H-K; $n=10$).

AHNP was induced by infusion of 5% TAC (1 mL/kg) into biliopancreatic duct. After models of AHNP were made, L-Arg (100 mg/kg) was immediately injected via inferior vena to make L-Arg-treated models. Sham-operated models were made by flipping ceca. Samples of liver and blood were taken for analysis.

Alanine aminotransferase, aspartate aminotransferase, AST, serum amylase, and NO concentrations in the liver

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were detected to assess the degrees of hepatic injuries. ALT and AST concentrations were measured using an automatic biochemistry analyzer. Concentrations of serum amylase and NO in the liver were measured spectrophotometrically.

TLR2/4 mRNA and TNF-α mRNA gene expression in the liver

The expression of TLR2/4mRNA and TNF-αmRNA in the liver was assayed by RT-PCR. The sequence of TLR2 primer was 5'-CGCTTCCTGA ACTTGTCC-3' (sense), 5'-GGTTGTCACCTGCTTCCA-3' (anti-sense) and 5'-ACTAAGAGGCGGAGCGGA-3' (fluorescent probe). The sequence of TLR4 primer was 5'-ATCATGGCATTGTTCCTTTCCT-3' (sense), 5'-CTGAGATTCTG ATCCATGCATTG-3' (anti-sense) and 5'-TCGGTAACG ACGGTTGTAG-3' (fluorescent probe). The sequence of TNF-α primer was 5'-CCCGTCG GAACAGGGAACTT-3' (sense), 5'-GGGTGTCCTTAGGGCAAG-3' (anti-sense) and 5'-CGAGGAGGCGAACCACCAA-3' (fluorescent probe). The sequence of β-actin primer 5'-GAACGGTGAAGGTGACAG-3' (sense), 5' -TAGA GAGAGTGGGGTGG-3' (anti-sense) and 5'-ACCACAGCACCTGCGG GAT-3' (fluorescent probe). Results were obtained using FTC-2000 real-time instrument (Fengling Biotechnology Limited Company, Shanghai, China).

Statistical analysis

The data were expressed as mean ± SD. The differences between the two groups were assessed by Student's *t*-test. $P<0.05$ was considered statistically significant.

RESULTS

Serum amylase, ALT and AST concentrations

Serum amylase concentration was lower in CQ-treated and L-Arg-treated groups than in AHNP group (*t*: 2.087-2.195, $P<0.05$). ALT and AST concentrations were low in sham-operated group but significantly increased in AHNP group (*t*: 4.58-9.740, $P<0.01$). Administration of CQ or L-Arg significantly reduced ALT and AST concentrations in the liver (*t*: 1.074-8.765, $P<0.05$, Table 1).

NO, TNF-α concentrations and TLR2/4mRNA expression

TNF-α was low in sham-operated group but significantly increased in AHNP group (*t*: 6.848-9.959, $P<0.01$). Administration of CQ or L-Arg significantly reduced TNF-α concentration in liver (*t*: 3.946-8.997, $P<0.01$). NO concentration was markedly lower in AHNP group than in sham-operated group (*t*: 2.403–8.521, $P<0.05$, CQ-treated group and L-Arg-treated group (*t*: 2.138-9.597, $P<0.05$). TLR2/4mRNA expression was low in sham-operated group but markedly increased at 3 h in AHNP group and peaked at 12 h (*t*: 2.193-9.623, $P<0.01$). TLR2/4mRNA expression was inhibited by CQ (*t*: 2.294-8.382, $P<0.01$), and L-Arg (*t*: 3.880-8.995, $P<0.05$; Table 2).

DISCUSSION

Ten members have been identified from the mammalian TLR family[5,6]. TLRs belong to a wider superfamily, called IL-1 receptors/TLR superfamily, including receptors for the pro-inflammatory cytokines IL-1 and IL-18. All members possess cytoplasmic Toll/IL-1 receptor (TIR) domains. The TIR domain consisting of 160 amino acids is essential for signaling. TLRs are the key front-line sensors of invading microbes, responding to a wide range of microbial products through recognizing a different pathogen-associated molecular pattern (PAMP). At the same time, when TLRs are combined with PAMP, antigen presenting cells (APCs) are activated and produce co-

Table 2 NO and TNF-α concentrations, and TLR2/4mRNA expression in livers (mean±SD)

		n	NO (μmol/gprot)	TNF-α	TLR2	TLR4
A	unit	10	16.19±0.862	0.003±0.129E-3	0.115E-5±0.229E-6	0.1145E-2±0.545E-4
B	unit	10	12.91±1.058[a]	2.331±0.101[b]	0.197E-2±0.114E-3[a]	0.175±0.349E-2[b]
C	unit	10	9.53±0.344[b]	1.618±0.173[b]	0.275E-2±0.352E-4[b]	0.285±0.516E-2[b]
D	unit	10	4.52±0.356[b]	0.296±0.04[b]	0.294E-2±0.998E-4[b,c]	2.673±2.795E-2[b]
E	unit	10	27.78±0.542[b,d]	1.440±5.147E-2[b,d]	1.037E-4±3.299E-6[b,d]	0.026±3.462E-3[b,d]
F	unit	10	20.73±0.462[b,d]	0.862±3.197E-2[b,d]	1.884E-4±4.679E-6[b,d]	0.108±6.115E-3[b,d]
G	unit	10	13.70±0.734[a,d]	0.117±1.492E-2[b,d]	2.443E-4±7.714E-6[b,d]	0.348±6.807E-3[b,d]
H	unit	10	9.87±0.095[b]	0.676±2.092E-2[b,d]	2.324E-2±0.532E-4[b,d]	0.229±6.883E-3[b,d]
I	unit	10	11.69±0.954[b,c,d]	0.369±1.300E-2[b,d]	0.210E-2±1.691E-4[b,c,d]	0.187±0.085E-3[b,d]
J	unit	10	25.43±0.919[b,d]	0.115±0.587E-2[b,d]	0.163E-2±0.404E-4[b,e]	0.107±1.946E-3[b,d]
K	unit	10	32.99±0.382[b,d]	0.058±0.652E-2[b,d]	0.100E-2±0.316E-4[b,e]	0.084±5.522E-3[b,d]

[a]$P<0.05$, [b]$P<0.01$ *vs* A unit; [c]$P>0.05$ *vs* ahead unit; [d]$P<0.01$, [e]$P<0.05$ *vs* unit at the same time point of AHNP group.

stimulatory molecules and cytokines, activating specific immune responses. TLR2/4 plays the most important role in responding to bacterial infections. TLR2 is the major receptor for PAMPs of Gram-positive bacteria, such as peptidoglycan and lipoteichoic acids. TLR4, the major receptor for LPS, are highly susceptible to infections with Gram-negative bacteria and fungal pathogens, such as lipopolysaccharide (LPS) and heat shock protein (HSP)[7]. Mechanisms responsible for TLR-mediated protection, potentiation of cytokine release, mediation of neutrophil recruitment to the site of infection and release of oxygen and nitrogen radicals, and contribute to TLR activation[8]. Researches showed that $TLR2^{-/-}$ or $TLR4^{-/-}$ mice have an increased susceptibility to infections[9-12]. NO produced from L-Arg by catalysis of nitric oxide synthase (NOS) is the only resource of NO in body[13]. In our experiment, rats were injected with L-Arg so that NO concentration in the bodies of rats was elevated. At the same time, it was reported that TLR2/4mRNA expression can be inhibited by CQ[14]. In the present study, we investigated the changes of TLR2/4 gene expression and NO concentration in the liver when TLR2/4mRNA expression was inhibited by CQ and the effect of NO on TLR2/4 gene expression in the liver of AHNP rats.

Enteric bacteria and endotoxin can result in aggravation of AHNP. In the course, enteric bacteria and endotoxin enter the liver through portal vein and are deactivated. But when the function of the liver is damaged or the quantity of enteric bacteria and endotoxin exceeds the endurance of liver, bacteria and endotoxin enter the blood and result in sepsis. At the same time, AHNP can lead to the dysfunction of the liver and even the failure of liver, suggesting that liver may play an important role in the pathogenesis and development of AHNP.

Our study showed that TLR2/4mRNA expression could be detected in sham-operated group (0.155E-5±0.230E-6 and 0.115E-2±0.545E-4), but markedly increased at 3 h in AHNP group (0.197E-2±0.114E-3 and 0.175±0.349E-2) and peaked at 12 h (0.294E-2±0.998E-4 and 2.673±2.795E-2, $P<0.01$). Hepatic injuries were aggravated, while TNF-α concentration increased and NO concentration decreased ($P<0.05$). When TLR2/4mRNA expression was inhibited by CQ (3 h: 1.037E-4±3.299E-6 and 0.026±3.462E-3; 6 h: 1.884E-4±4.679E-6 and 0.108±6.115E-3; 12 h: 2.443E-4±7.714E-6 and 0.348±6.807E-3; $P<0.01$), hepatic injuries were relieved while NO concentration increased and TNF-α concentration decreased ($P<0.05$). When AHNP rats were treated with L-Arg, TLR2/4 mRNA expression was effectively inhibited (50 mg-T: 0.232E-2±0.532E-4 and 0.230±6.883E-3; 100 mg-T: 0.210E-2±1.691E-4 and 0.187±0.849E-2; 200 mg-T: 0.163E-2±0.404E-4 and 0.107±0.195E-2; 400 mg-T: 0.100E-2±0.317E-4 and 0.084±0.552E-2; $P<0.01$) and hepatic injuries were relieved. At the same time, NO concentration markedly increased and TNF-α concentration decreased ($P<0.05$).

When TLR2/4mRNA expression inhibited, synthesis and release of inflammatory factors decreased and hepatic injuries were relieved, suggesting that lower concentration of NO has the anti-inflammatory effect[15,16]. In our study, NO concentration in AHNP rats was decreased. When TLR2/4mRNA expression was inhibited by CQ, NO concentration increased, suggesting that TLR2/4mRNA expressions can be inhibited by NO. Our results suggest that synthesis and release of anti-inflammatory factors might be inhibited by TLR2/4mRNA expression and TLR2/4mRNA gene expression might play an important role in the pathogenesis and development of hepatic injury in AHNP.

Lower concentration of NO may exert its anti-inflammatory effect by reducing neutrophilic leukocytes, platelet and adhesion molecules, concentration of inflammatory factors in bronchoalveolar lavage, improving the pancreatic blood flow and pancreatic microcirculation, inhibiting the production of oxyradicals and cytokines, interaction of leukocytes and endotheliocytes.

NO may decrease TLR2/4mRNA expression by directly inhibiting production of cytokines, reducing differentiation of phagocytes and interaction between leukocytes and endotheliocytes. The results of our experiment provide evidence for the role of NO in TLR2/4mRNA expression in the liver of AHNP rats.

REFERENCES

1 **Morrison CP**, Teague BD, Court FG, Wemyss-Holden SA,

Metcalfe MS, Dennison AR, Maddern GJ. Experimental studies of serum cytokine concentration following pancreatic electrolytic ablation. *Med Sci Monit* 2003; **9**: BR43-BR46

2 **Zhang Q**, Ni Q, Cai D, Zhang Y, Zhang N, Hou L. Mechanisms of multiple organ damages in acute necrotizing pancreatitis. *Chin Med J (Engl)* 2001; **114**: 738-742

3 **Maki Sugimoto**, Tadahiro Takada, Hideki Yasuda, Hodaka Amano, Masahiro Yoshida, Takahiro Isaka, Naoyuki Toyota, Keita Wada, Kenji Takagi. Cytokine storm in acute pancreatitis: influence of inflammatory attack and arterial infusion therapy. *International Congress Series* 2003; **1255**: 183-186

4 **Frantz S**, Kelly RA, Bourcier T. Role of TLR-2 in the activation of nuclear factor kappaB by oxidative stress in cardiac myocytes. *J Biol Chem* 2001; **276**: 5197-5203

5 **Takeda K**, Akira S. TLR signaling pathways. *Semin Immunol* 2004; **16**: 3-9

6 **Zhang D**, Zhang G, Hayden MS, Greenblatt MB, Bussey C, Flavell RA, Ghosh S. A toll-like receptor that prevents infection by uropathogenic bacteria. *Science* 2004; **303**: 1522-1526

7 **Netea MG**, Van der Meer JW, Kullberg BJ. Toll-like receptors as an escape mechanism from the host defense. *Trends Microbiol* 2004; **12**: 484-488

8 **Kaisho T**, Akira S. Toll-like receptors as adjuvant receptors. *Biochim Biophys Acta* 2002; **1589**: 1-13

9 **Echchannaoui H**, Frei K, Schnell C, Leib SL, Zimmerli W, Landmann R. Toll-like receptor 2-deficient mice are highly susceptible to Streptococcus pneumoniae meningitis because of reduced bacterial clearing and enhanced inflammation. *J Infect Dis* 2002; **186**: 798-806

10 **Koedel U**, Angele B, Rupprecht T, Wagner H, Roggenkamp A, Pfister HW, Kirschning CJ. Toll-like receptor 2 participates in mediation of immune response in experimental pneumococcal meningitis. *J Immunol* 2003; **170**: 438-444

11 **Woods JP**, Frelinger JA, Warrack G, Cannon JG. Mouse genetic locus Lps influences susceptibility to Neisseria meningitidis infection. *Infect Immun* 1988; **56**: 1950-1955

12 **Shahin RD**, Engberg I, Hagberg L, Svanborg Edén C. Neutrophil recruitment and bacterial clearance correlated with LPS responsiveness in local gram-negative infection. *J Immunol* 1987; **138**: 3475-3480

13 **Li GK**, Zhang ZHD, Liu XB, Guo YY. The role of L-arginine in the treatment of acute edematous pancreatitis in rats. *Chin Experimental Surgery* J 1999; **16**: 244-248

14 **Hong Z**, Jiang Z, Liangxi W, Guofu D, Ping L, Yongling L, Wendong P, Minghai W. Chloroquine protects mice from challenge with CpG ODN and LPS by decreasing proinflammatory cytokine release. *Int Immunopharmacol* 2004; **4**: 223-234

15 **Chen XJ,** Zhang QY, Chen BC. Experimental research of L-argignine in the treatment of acute hemorrhagic necrotizing pancreatitis in early stage. *J Hepatopancreatobiliary Surg* 2002; **14**: 220-222

16 **Masamune A**, Shimosegawa T, Satoh A, Fujita M, Sakai Y, Toyota T. Nitric oxide decreases endothelial activation by rat experimental severe pancreatitis-associated ascitic fluids. *Pancreas* 2000; **20**: 297-304

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CASE REPORT

Retention mucocele of distal viable remnant tip of appendix: An unusually rare late surgical complication following incomplete appendectomy

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Abstract

A 67-year old man was presented with a 6-mo history of recurrent right lower quadrant abdominal pain. On physical examination, a vague mass was palpable in the right lumbar region. His routine laboratory tests were normal. Ultrasonography showed a hypoechoic lesion in the right lumbar region anterior to the right kidney with internal echoes and fluid components. Abdominal contrast-enhanced computed tomography (CECT) showed a well-defined hypodense cystic mass lesion lateral to the ascending colon/caecum, not communicating with the lumen of colon/caecum. After complete open excision of the cystic mass lesion, gross pathologic examination revealed a turgid cystic dilatation of appendiceal remnant filled with the mucinous material. On histopathological examination, mucinous cyst adenoma of appendix was confirmed. We report this rare unusual late complication of mucocele formation in the distal viable appendiceal remnant, which was leftover following incomplete retrograde appendectomy. This unusual complication is not described in the literature and we report it in order to highlight the fact that a high index of clinical and radiological suspicion is essential for the diagnosis of mucocele arising from a distal viable appendiceal remnant in a patient who has already undergone appendectomy presenting with recurrent abdominal pain.

Key words: Retention mucocele; Appendix; Incomplete appendectomy; Surgical complication

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INTRODUCTION

Appendiceal mucocele was first described by Rokitanski in 1842[1] and an unusual variant of appendiceal mucocele -myxoglobulosis was first described by Latham in 1897[1-3]. The term "mucocele of appendix"[4,5] is an inherently imprecise descriptive term that refers to any macroscopic (localised or diffuse) globular cystic dilatation of appendix (unilocular or multilocular) filled with thick tenacious mucoid/mucinous material regardless of underlying cause and is not a pathologic entity.

The majority of mucoceles of appendix arise secondary to proximal obstruction of appendiceal lumen in which the appendiceal lumen is usually in communication with caecum[6]. The potential causes of proximal appendiceal obstruction include faecolith, epithelial/mucosal hyperplasia, post inflammatory-fibrosis, cystadenoma, cystadenocarcinoma, carcinoid tumor, endometriosis and developmental anomalies such as occlusive membrane or obstructive diaphragm at the level of appendiceal orifice[7]. But the origin of mucocele of appendix from a distal viable leftover remnant tip of appendix not communicating with the caecum following incomplete retrograde open appendectomy has not been described.

We report such an extremely rare case of retention mucocele arising from a distal vascularized remnant of appendix tip, which is a leftover following incomplete open appendectomy.

CASE REPORT

We report a 67-year old man who was presented with a 6-mo history of recurrent right lower quadrant abdominal pain not associated with vomiting. He had normal appetite and bowel habits. He was not a hypertensive and diabetic, neither an alcoholic nor a smoker. He underwent open appendectomy 15 years ago. At presentation his vital

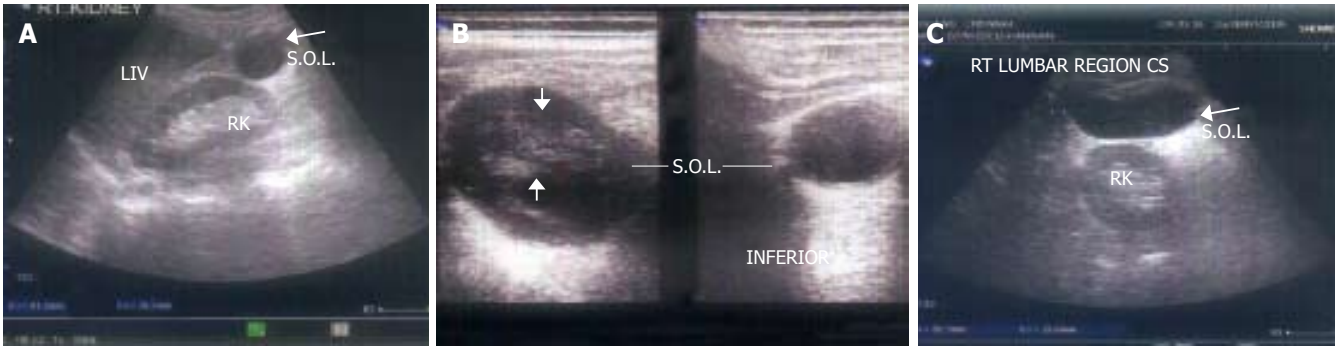

Figure 1 Ultrasonography shows a hypoechoeic space occupying lesion measuring 6.5 cm × 3 cm × 6 cm in the right lumbar region anterior to right kidney (arrows). Internal echoes and fluid components are seen within the cystic mass (arrowheads) (**A**-**C**).

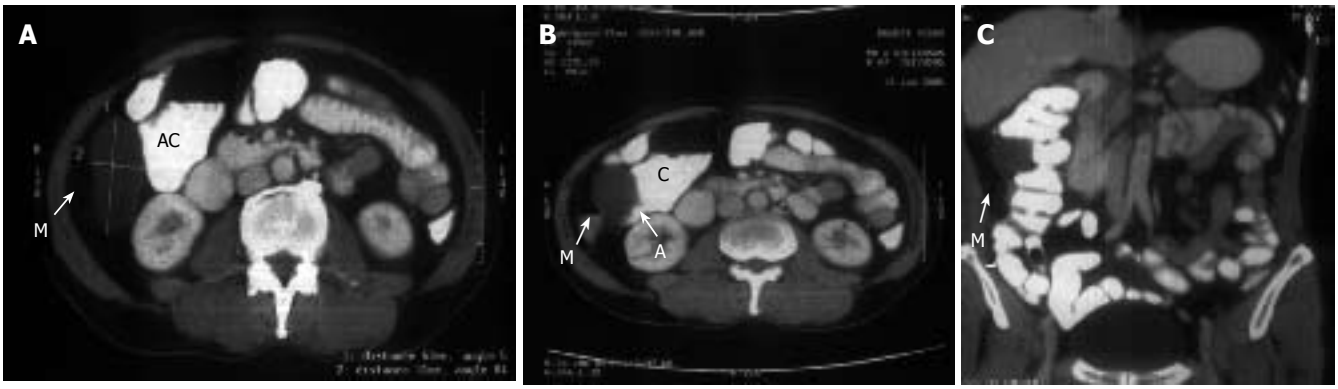

Figure 2 CECT shows a well defined hypodenseblind ending tubular lesion (arrows) measuring 6.8cm × 3.1cm × 3.0cm (CT value + 32HU) in the right lumbar region lateral to ascending colon along the antimesenteric border not communicating with the lumen of colon(**A**-**C**). C=Caecum, AC=Ascending colon, M=Mucocele, A=Distal appendiceal remnant not communicating with caecal lumen.

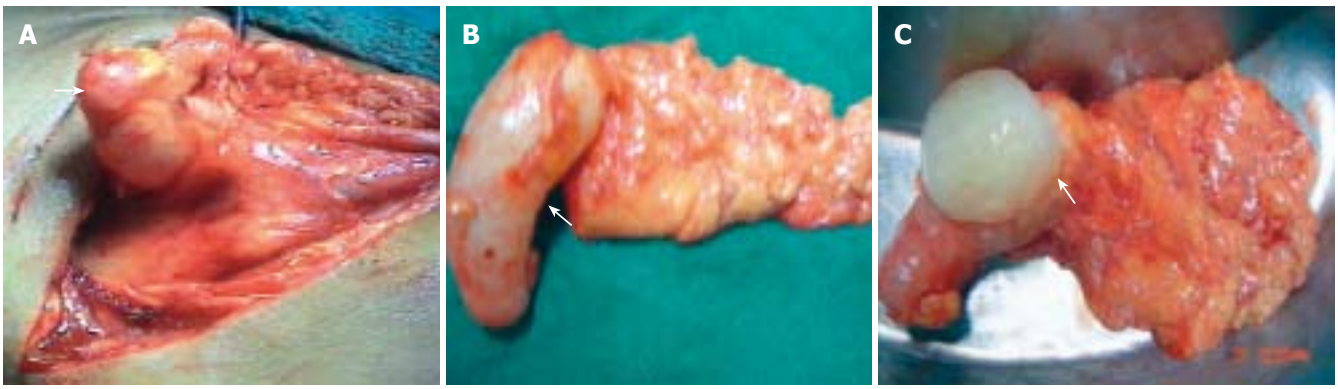

Figure 3 Macroscopic/gross pathology. **A**: Perioperative photograph shows a turgid cystic mass measuring 6cm × 5cm × 3cm in the distal appendicular remnant with a separate mesentery located adjacent to the pulled up caecum and not communicating with lumen of caecum (arrows); **B**: Resected specimen shows cystic dilatation of turgid distal appendicular remnant with surrounding fibrofatty, mesenteric, and omental tissues (arrow); **C**: Cut open resected specimen shows protruding mucinous material from the lumen of cystically dilated turgid distal appendicular remnant (arrow).

parameters and systemic examination were normal. On examination of abdomen, a vague mass was palpable in the right lumbar region suspicious of a retroperitoneal tumor. Rectal examination was normal.

Hemoglobin value was 13.8 gm/dL(reference range: 13.5-17.0 gm/dL) and other biochemical investigations, chest X-ray and electro-cardiogram were normal. Upper gastrointestinal (GI) endoscopy and colonoscopy study was normal. Ultrasonography revealed a 6.5cm×3cm×3.5cm hypoechoic space-occupying lesion in the right lumbar region anterior to the right kidney with internal echoes and fluid components which suggested retroperitoneal cyst (Figure 1). Abdominal CECT showed a well defined blind ending tubular hypodense lesion with CT value +32 Hounsfield unit (HU) in the right lumbar region lateral to the ascending colon along the antimesenteric border (Figure 2). The lesion was not communicating with lumen of the colon, which suggested colonic duplication cyst.

At laparotomy, a 6cm×3cm mucus-filled turgid mass was found in the distal appendiceal remnant having a

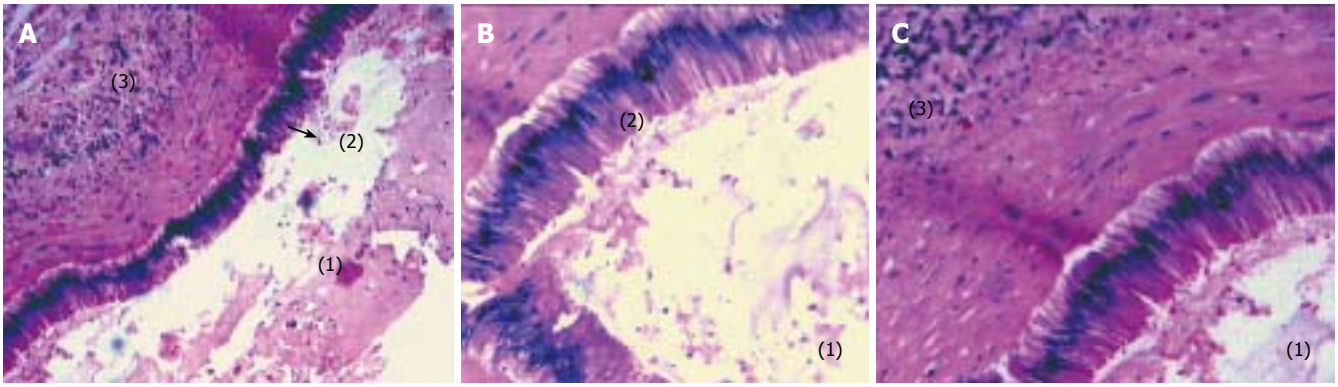

Figure 4 Histopathological microphotographs of mucinous cystadenoma of appendix. **A**: Low power microscopic view. Appendicular lumen shows mucinous secretions (1) with no epithelial elements. Mucosa lined by a single layer of mucinous columnar epithelium with basally situated nuclei (2) does not show any atypia with underlying lymphoid aggregatae (3). **B**,**C**: High power microscopic view. Appendiceal lumen shows lakes of mucin (1) with no epithelial elements. Mucosa lined by a single layer of mucinous columnar epithelium with basally situated nuclei (2) does not show any atypia with underlying lymphoid aggregates(3).

separate mesentery with no communication with the ceacum (Figure 3A). Complete surgical excision was performed with an uneventful recovery. Gross pathological examination showed a distended turgid appendiceal remnant filled with characteristic mucinous material (Figures 3B, 3C). Histopathological examination showed that the appendix was lined by a single layer of mucinous epithelium with basally situated nuclei which did not show any atypia with underlying lymphoid aggregates. The appendiceal lumen showed mucinous secretions (lakes of mucin) with no epithelial elements suggestive of mucinous cyst adenoma-appendix with no evidence of malignancy or dysplasia. (Figures 4A,4B,4C). The patient was symptom-free during a 6-mo follow-up period.

DISCUSSION

Primary appendiceal mucocele, a relatively uncommon clinical entity, is most frequently an incidental finding at the time of surgery and is occasionally discovered only at pathological examination[8]. The majority of these patients are not diagnosed preoperatively and in fact 60%[8] of them are diagnosed incidentally during surgery for some other disease. The incidence of appendiceal mucocele is estimated to be 0.2-0.3% of appendicectomy specimens with myxoglobulosis constituting 0.35-0.8% of mucoceles[7].

Mucoceles are histologically subdivided into four types on the basis of World Health Organization classification[4, 8].

Simple/non neoplastic mucocele or retention mucocele or obstructive form of mucocele[5] is defined as cystic dilatation of the distal appendix with accumulation of abnormal mucoid material in the appendiceal lumen secondary to appendiceal outflow obstruction.

Benign neoplastic mucocele-mucinous cystadenoma[5] is defined as dilated mucus /mucin filled appendix containing adenomatous mucosa lined by atypical mucinous epithelium containing basal nuclei and showing only minimal dysplastic features. Secondary changes[9] in mucinous cyst adenoma include thinning of the wall, extensive ulceration, calcification and ossification ("porcelain appendix")[5]. There is also a high association between appendiceal mucinous cystadenoma with ovarian mucinous cyst adenoma and synchronous or metachronous neoplasms elsewhere in the colon[10].

Malignant mucocele[5]-mucinous cystadenocarcinoma is defined as adenocarcinoma associated with mucus-filled cystic dilatation of the appendix presenting as mucocele. A malignancy is suspected at surgery in about 30%[4-5]. In the others the diagnosis is made during pathologic examination.

Cystadenocarcinoma[4] is grossly indistinguishable from a cystadenoma. But histologically[5] the former is distinguished from mucinous cystadenoma by three criteria: presence of invasive neoplasm below the level of muscularis mucosa, when the muscularis mucosa cannot be distinguished because of distortion or fibrosis, the diagnosis is made by the finding of infiltrative tongues of tumor or single tumor cells in the wall (infiltrative appearance of border of epithelial elements) as opposed to the broad pushing edge appearance of the borders of epithelial elements that characterize mucinous tumor of uncertain/undetermined malignant potential (UMP)[5,11], presence of malignant epithelial cells in the lakes of mucin either in the wall of the appendix or outside the appendix.

Myxoglobulosis or Caviar appendix[5,7] is an extremely rare variant of appendiceal mucocele caused by proximal obstruction of appendiceal lumen in which pieces of mucinous/mucoid material can become broken off the appendiceal wall into the appendiceal lumen resulting in the formation of characteristic pearl-like translucent "mucinous globules" or pearly luminal spheroids[7] or a "cluster of frog eggs"[12] 1-10 mm in diameter with surface calcification.

Mucocele of appendix is most common in the sixth or seventh decade of life with a female preponderance[7]. The common presenting symptoms of appendiceal mucocele are episodic right lower quadrant abdominal pain (27%)[8], abdominal mass (16%)[8], weight loss (10%)[8] and change in bowel habits (5%)[8]. Complications[12-13] of mucocele include intussusception, bleeding, perforation, peritonitis, rupture and pseudomyxoma peritonei.

Colonoscopy may show a smooth glassy submucosal

or extra-mucosal caecal mass moving in and out with respiratory movement. This endoscopic sign has been described as the "trapped balloon sign"[14]. The classical CT scan findings[7, 15] of a mucocele in a patient who has not undergone appendectomy are a cystic low attenuation well encapsulated round or ovoid mass with smooth regular walls in the right lower quadrant adherent to caecum, mural calcification in the wall of the mucocele, and absence of peri-appendiceal inflammation or abscess which is the key differentiating point in excluding acute appendicitis. The presence of thickened wall and enhanced nodules favors the diagnosis of mucinous cystadenocarcinoma.

The clinical and radiological differential diagnosis[7,13] of mucocele of appendix includes mesenteric cyst, colonic duplication cyst, colonic lymphoma and lipoma, intussusception , right ovarian cyst and hydrosalphinx.

The treatment of mucocele of appendix is essentially by simple appendectomy but in cases of rupture or suspected malignancy a standard right hemicolectomy is indicated[8]. At the time of surgery, a spontaneous appendiceal perforation or any extravasation from appendicular lumen is strongly suggestive of malignancy in such situations, a right hemicolectomy[8,16] should always be performed with a curative intent. Laparoscopic appendectomy for mucocele removal has been described, but caution has also been suggested because of the risk of port site recurrences[17].

In a patient who has undergone open appendectomy, with the CT finding of a cystic well encapsulated mass in the right lumbar region adherent to the pulled up caecum/ ascending colon not communicating with the lumen of colon/caecum, one should consider the possibility of mucocele of distal appendiceal remnant. Therefore, a high index of clinical and radiological suspicion is essential for the preoperative diagnosis of mucocele of distal appendiceal remnant in a patient who has undergone appendectomy.Incomplete surgical removal of appendix must be avoided in order to prevent the late complication of mucocele formation in the distal leftover vascularized remnant tip of appendix. All appendiceal mucoceles measuring at least 2 cm must be completely excised to eliminate the chance of progression to malignancy. The association between appendiceal mucocele and colonic neoplasm is more clear and logical to recommend surveillance colonoscopy in patients with diagnosis of appendiceal mucocele, at least in those with appendiceal mucinous cystadenoma.

REFERENCES

1 **Gonzalez JE**, Hann SE, Trujillo YP. Myxoglobulosis of the appendix. *Am J Surg Pathol* 1988; **12**: 962-966
2 **Lubin J**, Berle E. Myxoglobulosis of the appendix. Report of two cases. *Arch Pathol* 1972; **94**: 533-536
3 **Rolón PA**. Myxoglobulosis of the appendix. *Int Surg* 1977; **62**: 355-356
4 **Appelman HD**. Epithelial neoplasia of the appendix. In: Norris HT, ed. Pathology of the colon, small intestine, and anus 2nd ed. New York: Churchill Livingstone pub, 1991: 263-303
5 **Parakrama Chandrasoma**. Gastrointestinal Pathology. 1st ed. Appleton& Lange pub, 1999: 239-241
6 **Stacey E**. Mills. Sternberg's Diagnostic Surgical Pathology. 4th ed. Lippincott Willams/Wilkins pub, 2004: 1576-1599
7 **Viswanath YK**, Griffiths CD, Shipsey D, Oriolowo A, Johnson SJ. Myxoglobulosis, a rare variant of appendiceal mucocele, occurring secondary to an occlusive membrane. *J R Coll Surg Edinb* 1998; **43**: 204-206
8 **Stocchi L**, Wolff BG, Larson DR, Harrington JR. Surgical treatment of appendiceal mucocele. *Arch Surg* 2003; **138**: 585-59; discussion 585-59
9 Juan Rosai – Rosai and Ackerman's Surgical pathology. 9th ed. Vol I. Mosby pub, 2004: 761– 765
10 **Seidman JD**, Elsayed AM, Sobin LH, Tavassoli FA. Association of mucinous tumors of the ovary and appendix. A clinicopathologic study of 25 cases. *Am J Surg Pathol* 1993; **17**: 22-34
11 **Isaacs KL**, Warshauer DM. Mucocele of the appendix: computed tomographic, endoscopic, and pathologic correlation. *Am J Gastroenterol* 1992; **87**: 787-789
12 **Dachman AH**, Lichtenstein JE, Friedman AC. Mucocele of the appendix and pseudomyxoma peritonei. *AJR Am J Roentgenol* 1985; **144**: 923-929
13 **Huang HC**, Liu TP, Jeng KS. Intussusception of mucocele of the appendix: a case report. *Zhonghua Yi Xue Za Zhi (Taipei)* 1994; **53**: 120-123
14 **Raijman I**, Leong S, Hassaram S, Marcon NE. Appendiceal mucocele: endoscopic appearance. *Endoscopy* 1994; **26**: 326-328
15 **Zissin R**, Gayer G, Kots E, Apter S, Peri M, Shapiro-Feinberg M. Imaging of mucocoele of the appendix with emphasis on the CT findings: a report of 10 cases. *Clin Radiol* 1999; **54**: 826-832
16 **Nitecki SS**, Wolff BG, Schlinkert R, Sarr MG. The natural history of surgically treated primary adenocarcinoma of the appendix. *Ann Surg* 1994; **219**: 51-57
17 **González Moreno S**, Shmookler BM, Sugarbaker PH. Appendiceal mucocele. Contraindication to laparoscopic appendectomy. *Surg Endosc* 1998; **12**: 1177-1179

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CASE REPORT

TIPSS for variceal hemorrhage after living related liver transplantation: A dangerous indication

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Abstract

The introduction of transjugular intrahepatic portal-systemic stent-shunt (TIPSS) has been a major breakthrough in the treatment of portal hypertension, which has evolved to a large extent, into a routine procedure. A 21-year-old male patient with progressive graft fibrosis/cirrhosis requiring TIPSS for variceal hemorrhage in the esophagus due to portal hypertension was unresponsive to conventional measures two years after living related liver transplantation (LDLT). Subsequently, variceal hemorrhage was controlled, however, liver function decreased dramatically with consecutive multi organ failure. CT scan revealed substantial necrosis in the liver. The patient underwent successful "high urgent" cadaveric liver transplantation and was discharged on postoperative d 20 in a stable condition.

Key words: Portal hypertension; Liver necrosis; Fibrosis; Cirrhosis

Schemmer P, Radeleff B, Flechtenmacher C, Mehrabi A, Richter GM, Büchler MW, Schmidt J. TIPSS for variceal hemorrhage after living related liver transplantation: A dangerous indication. *World J Gastroenterol* 2006; 12(3): 493-495

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INTRODUCTION

Transjugular intrahepatic portal-systemic stent-shunt (TIPSS) is defined as artificial shunts between branches of the portal vein and the systemic circulation within the liver parenchyma, and is comparable to a surgical H-shunt with partial decompression of portal hypertension. The first TIPSS has been performed by Rösch *et al*[1] in 1969 in dogs. After the stage had been set for the implantation of stent-shunts into human beings, the first TIPSS was performed on a 49-year-old male patient with liver cirrhosis and bleeding from esophageal varices due to portal hypertension[2]. Since then this procedure has rapidly progressed to a well defined and established standard procedure for a wide variety of liver disorders, i.e., TIPSS is preferred for acute or recurrent variceal hemorrhage refractory to medical treatment, endoscopic sclerotherapy or banding[3]. Since TIPSS enables pre-transplant patients to recover from bleeding episodes and improves their general status before a graft becomes available, this procedure forms a bridge to liver transplantation[4]. The first 30-d mortality after TIPSS (up to 11%) is usually due to liver failure, acute respiratory distress syndrome, sepsis, recurrent hemorrhage, or right heart or multi organ failure[4]. Here we report a case of liver necrosis with subsequent organ failure making it necessary for high urgency (HU) cadaveric liver transplantation following TIPSS for conventionally uncontrolled variceal hemorrhage after living related liver transplantation (LDLT).

CASE REPORT

A 21-year-old male patient underwent LDLT with biliodigestive anastomosis because of liver cirrhosis due to hereditary tyrosinemia type I and hepatocellular carcinoma ($T_3N_0M_0$). After an uneventful transplantation, the postoperative course was further complicated by a bilioma followed by both a biliocutaneous fistula and chronic cholangitis. Two years after LDLT, the patient was referred to our institution and presented with progressive cholestasis, all signs of portal hypertension (i.e., varicose veins of the esophagus and hypersplenism) while graft function was still adequate, classified as Child-Pugh A. Post-contrast sequences of MRI originally performed to identify the total extent of the biliocutaneous fistula, showed perfusion of the liver was inhomogeneous with regular appearance of the intra- and extrahepatic portal venous branches, inferior vena cava (IVC) and hepatic artery (Figure 1A). For a complete

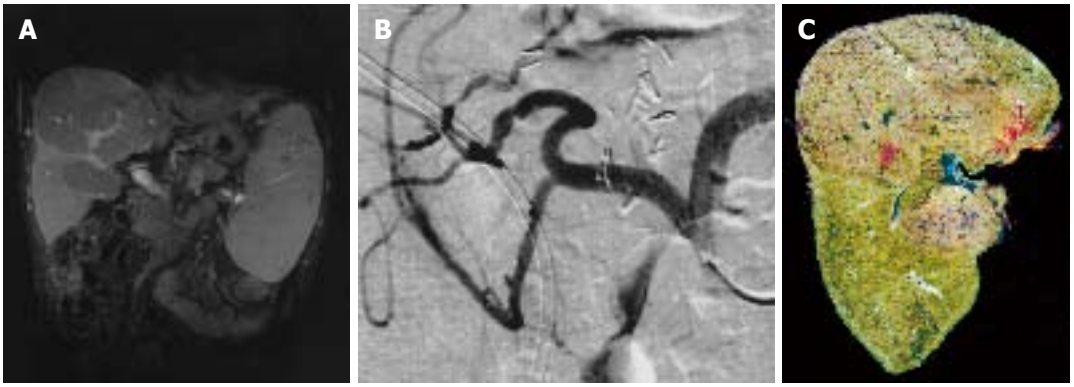

Figure 1 Pre-TIPSS MRI/ angiography and post-TIPSS macroscopy of the liver. Pictures illustrating pre-TIPSS inhomogeneous intrahepatic parenchyma texture (**A**); clinically irrelevant stenosis of the hepatic artery (**B**) and macroscopic aspect of the liver after TIPSS and organ failure due to substantial necrosis in areas where circulation was compromised before TIPSS (**C**).

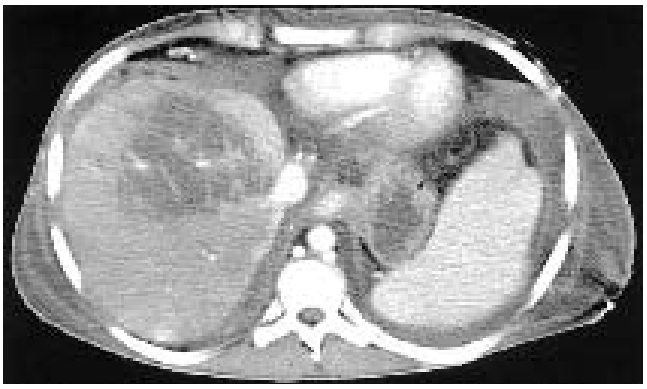

Figure 2 TIPSS-placement. Picture illustrating correctly placed TIPSS.

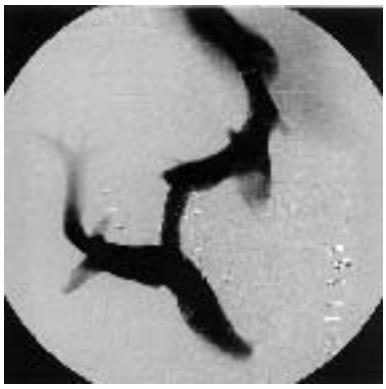

Figure 3 Spiral-CT scan after TIPSS was established. Spiral-CT after TIPSS revealed substantial necrosis at the time of liver failure.

examination of the patient's preoperative status, a celiac-mesenteric angiography was performed which identified a stenosis of the arterial anastomosis, considered as hemodynamically irrelevant (Figure 1B). Revision of the biliodigestive anastomosis was performed and a y-Roux hepatojejunostomy after adhesiolysis was established to close the biliocutaneous fistula, which maintained cholangitis. Histology of the intraoperative biopsy revealed severe fibrosis associated with secondary biliary cirrhosis of the transplant. After surgery the patient recovered quickly; however, on postoperative d 10 recurrent variceal hemorrhage unresponsive to conventional measures, including endoscopic therapy, required TIPSS since surgery was not feasible at that time. TIPSS was performed in a standard procedure with an uncoated 8/39 Corinthian-Stent, which decreased the portacaval pressure gradient from 23 mmHg (measured after a 8 mm tract was established) to 8 mmHg at the end of the procedure (Figure 2). Subsequently, variceal hemorrhage in the esophagus stopped, however, serum transaminases, bilirubin and ammonia increased, while liver function decreased dramatically with fulminant consecutive multi organ failure within 3 postoperative days. At that time, a spiral-CT scan revealed both substantial disturbances of intrahepatic perfusion and necrosis, while TIPSS, extrahepatic portal vein, hepatic artery and IVC displayed normal (Figures 2 and 3). One day later, the patient underwent successful (HU) orthotopic cadaveric re-transplantation of the liver and was discharged in a stable condition thereafter on postoperative d 20.

DISCUSSION

Biliary complications after LDLT are observed in 15-40% of patients, which can increase the risk of developing secondary biliary cirrhosis and all signs of portal hypertension (i.e. variceal bleeding)[5]. The significant decrease of portal flow to the liver after TIPSS, due to portal decompression, may result in decreased liver function. An increase in hepatic arterial flow, however, maintains sufficient liver function in most patients[9]. The latter is inadequate in a small number of patients and TIPSS results in liver failure[4]. Further, cirrhotic patients have a cirrhotic cardiomyopathy. This is important since TIPSS shunts a large blood volume back to the right heart and may impair cardiac function.

Till date most information of both arterial and portal venous hemodynamic in the liver are based on indirect scintigraphic or invasive electromagnetic measurement of blood flow during portosystemic shunt surgery. Burchell *et al*[6] demonstrated in their early work an increased blood flow in the hepatic artery after porto-caval shunting. Since no flow measurements were performed intravascularly, precise information on complex flow changes over time (i.e., detection of mechanisms of arterio-portal compensation) were impossible[6]. In the late sixties Hanson[7] and Lutz[8] hypothesized that both dilatation and contraction of the intrahepatic arterioles, which occur immediately after liver- or shunt-operations, can be defined as regulatory reflex-mediated responses to changes of pressure in the hepatic sinusoids. Richter *et al*[9] later demonstrated

online the dynamic changes of arterial and portal blood flow during partial porto-systemic decompression with TIPSS. Real-time monitoring of the arterial flow with endoluminal catheters revealed that arterial blood flow increased significantly and momentarily while portal venous flow decreased; however, total liver perfusion after TIPSS maintained steady in their studies. Since the fraction of arterial perfusion was increased after TIPSS, both pressure of portal venous perfusion and portal flow in hepatic sinusoids decreased. Reduced portal venous inflow in hepatic capillaries was compensated by an increased arterial perfusion. Since these changes in hepatic flow were immediately present in all the patients after TIPSS, underlying regulatory mechanisms that maintain a constant sinusoidal liver perfusion are most likely based on reflexes.

How can it be explained that major disturbances in hepatic circulation with subsequent liver necrosis and deterioration of liver function occurred after TIPSS in the case described above? Several factors most likely contributed to pathology: One major factor was the initially compensated stenosis of the hepatic artery, diagnosed with a celiac-mesenteric angiography (Figure 1B), which most likely prevented an increase in the arterial flow reserve to compensate for a decreased portal flow after TIPSS. Moreover, chronic inflammation (cholangitis) and liver cirrhosis may have also factored into the reduction of possible regulatory mechanisms stated above that compensated for reduced portal flow[10,11]. Therefore, severe dysfunction of the liver due to disturbances in hepatic perfusion, with subsequent necrosis of liver tissue, complicated treatment after TIPSS (Figures 1C and 3). In such cases, TIPSS should be indicated with great caution in centers where the salvage therapy, high urgent liver transplantation, can be performed.

REFERENCES

1 **Rösch J**, Hanafee WN, Snow H. Transjugular portal venography and radiologic portacaval shunt: an experimental study. *Radiology* 1969; **92**: 1112-1114
2 **Richter GM**, Palmaz JC, Nöldge G, Rössle M, Siegerstetter V, Franke M, Wenz W. [The transjugular intrahepatic portosystemic stent-shunt. A new nonsurgical percutaneous method]. *Radiologe* 1989; **29**: 406-411
3 **Rösch J**, Keller FS. Transjugular intrahepatic portosystemic shunt: present status, comparison with endoscopic therapy and shunt surgery, and future prospectives. *World J Surg* 2001; **25**: 337-45; discussion 345-6
4 **Barton RE**, Rösch J, Saxon RR, Lakin PC, Petersen BD, Keller FS. TIPS: short and long-term results: a survey of 1750 patients. *Sem Interv Radiol* 1995; **12**: 364-369
5 **Shiffman ML**, Brown RS, Olthoff KM, Everson G, Miller C, Siegler M, Hoofnagle JH. Living donor liver transplantation: summary of a conference at The National Institutes of Health. *Liver Transpl* 2002; **8**: 174-188
6 **Burchell AR**, Moreno AH, Panke WF, Nealon TF. Hepatic artery flow improvement after portacaval shunt: a single hemodynamic clinical correlate. *Ann Surg* 1976; **184**: 289-302
7 **Hanson KM**, Johnson PC. Local control of hepatic arterial and portal venous flow in the dog. *Am J Physiol* 1966; **211**: 712-720
8 **Lutz J**, Peiper U, Bauereisen E. [Appearance and size of veno-vasomotor reactions in liver circulation]. *Pflugers Arch Gesamte Physiol Menschen Tiere* 1968; **299**: 311-325
9 **Richter GM**, Brado M, Simon C, Mädler U, Radeleff B, Roeren T, Sauer P, Kauffmann GW. [Changes in liver perfusion caused by transjugular intrahepatic stent shunt (TIPSS)]. *Zentralbl Chir* 1997; **122**: 108-116
10 **Bader TR**, Braga L, Beavers KL, Semelka RC. MR imaging findings of infectious cholangitis. *Magn Reson Imaging* 2001; **19**: 781-788
11 **Revelon G**, Rashid A, Kawamoto S, Bluemke DA. Primary sclerosing cholangitis: MR imaging findings with pathologic correlation. *AJR Am J Roentgenol* 1999; **173**: 1037-1042

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CASE REPORT

Extensive retroperitoneal and right thigh abscess in a patient with ruptured retrocecal appendicitis: An extremely fulminant form of a common disease

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Abstract

As a disease commonly encountered in daily practice, acute appendicitis is usually diagnosed and managed easily with a low mortality and morbidity rate. However, acute appendicitis may occasionally become extraordinarily complicated and life threatening. A 56-year-old man, healthy prior to this admission, was brought to the hospital due to spiking high fever, poor appetite, dysuria, progressive right flank and painful swelling of the thigh for 3 d. Significant inflammatory change of soft tissue was noted, involving the entire right trunk from the subcostal margin to the knee joint. Painful disability of the right lower extremity and apparent signs of peritonitis at the right lower abdomen were disclosed. Laboratory results revealed leukocytosis and an elevated C-reactive protein level. Abdominal CT revealed several communicated gas-containing abscesses at the right retroperitoneal region with mass effect, pushing the duodenum and the pancreatic head upward, compressing and encasing inferior vena cava, destroying psoas muscle and dissecting downward into the right thigh. Laparotomy and right thigh exploration were performed immediately and about 500 mL of frank pus was drained. A ruptured retrocecal appendix was the cause of the abscess. The patient fully recovered at the end of the third post-operation week. This case reminds us that acute appendicitis should be treated carefully on an emergency basis to avoid serious complications. CT scan is the diagnostic tool of choice, with rapid evaluation followed by adequate drainage as the key to the survival of the patient.

Key words: Acute appendicitis; Retrocecal appendicitis; Complication; Retroperitoneal abscess; Thigh abscess

INTRODUCTION

Acute appendicitis is a disease commonly encountered in daily practice, and a very low morbidity and mortality rate can be achieved with proper diagnosis and management at present[1,2]. Generally, a non-perforated acute appendicitis can be managed by urgent appendectomy, while perforated appendicitis which may be associated with the formation of localized abscess in the right iliac fossa or in the pelvic cavity, can be managed depending on their symptoms either by early appendectomy or by interval appendectomy following percutaneous drainage[3]. Even with a more severe form of ruptured appendicitises such as those complicated with diffuse peritonitis as commonly encountered in patients of pre-school age, the post-operative recovery is usually smooth[4]. However, acute appendicitises such as those forming appendiceal masses and extensive abscesses or resulting in intestinal obstruction may sometimes become more complicated and require a prolonged treatment period[3,4]. These complications should not be overlooked in order to avoid further sequelae. Hence, we present here a rare and critical case of ruptured retrocecal acute appendicitis with extensive formation of retroperitoneal and thigh abscess, re-emphasizing the importance of early diagnosis and prompt management for this common disease.

CASE REPORT

A 56-year-old man was generally in good health when he first experienced right flank and right thigh pain with daily spiking fever of up to 39 °C for 3 d. He also experienced progressive loss of appetite, epigastric fullness and painful disability of the right thigh. There was minimal abdominal pain initially, but this became more prominent over the right lower quadrant a few days later. He was brought to our hospital 3 d after the onset of the symptoms.

Physical examination on admission revealed an acute ill-

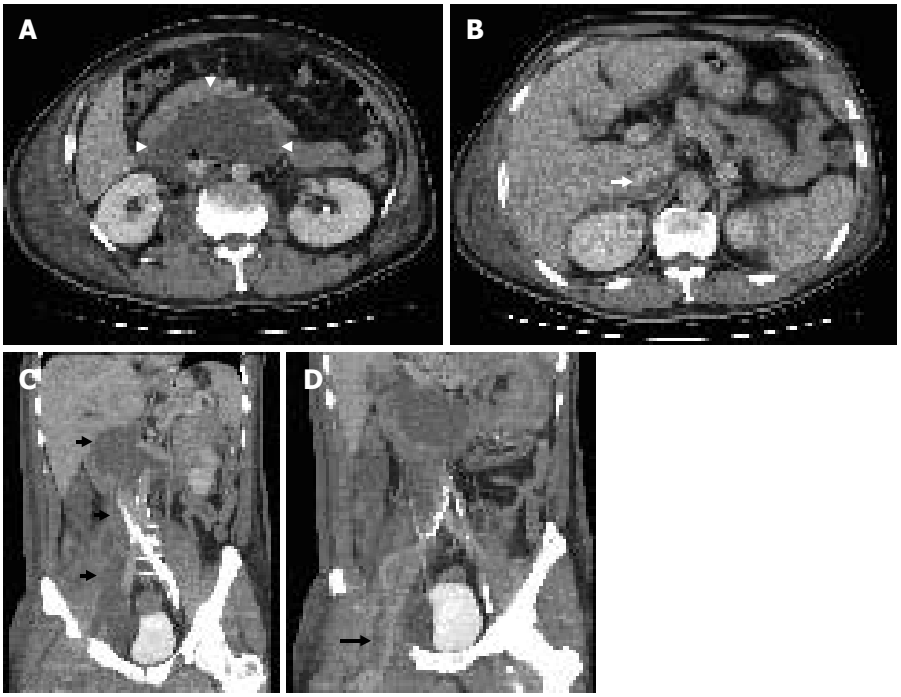

Figure 1 Retroperitoneal and right thigh abscess in a patient with ruptured retrocecal appendicitis. **A:** Retroperitoneal abscess with mass effect, demonstrating the upward-pushed duodenum and pancreatic head as well as compressed and encased inferior vena cava (white arrowheads); **B:** suspicious septic thrombus inside inferior vena cava (white arrow); **C:** reconstructed coronal image demonstrating extensive involvement of the abscess (short black arrows); **D:** dissection of the abscess in the right thigh via the femoral canal just beneath the femoral vessels (long black arrow).

looking man with a body temperature of 39.6 °C. He was slightly anemic in appearance and breathed both shallowly and rapidly at a rate of 28/min. He was lying in supine position with his right knee joint mildly flexed and hip joint externally rotated, being reluctant to move his right leg because of severe tenderness. A positive psoas stretch test was performed and the results indicated that there were significant inflammatory signs such as local heat, swelling, edema, and tenderness disclosed at areas involving the entire right trunk from right subcostal region to right knee joint, but no subcutaneous emphysema or crepitation was noted. Palpation of the abdomen revealed tenderness at right lower quadrant without muscle rigidity and no mass-like lesion was palpable. Laboratory data indicated leukocytosis with a WBC count of 16.4×10^6/mL, of which 80% were mature neutrophils and 3% were immature neutrophils. The hemoglobin level was slightly decreased to the level of 12.8 g/dL and his platelet count was also decreased to 617×10^6/mL. The C-reactive protein level was 26.1 mg/dL. All the other blood chemistry data were within the normal range. X-ray of the abdomen revealed an indistinct shadow of right psoas muscle. CT scan of the abdomen revealed formation of multiple gas-containing abscesses involving the entire right retroperitoneum with mass effect. At the upper abdominal region, the duodenum and pancreatic head were pushed upward by the abscess, the right perinephric space was filled with the abscess and the inferior vena cava at the same level was encased and compressed. There was also a suspicious septic thrombus inside the inferior vena cava. In addition to destroying the right psoas muscle, the abscess also dissected downward to the right thigh through the femoral canal, forming an abscess between muscle groups (Figure 1).

The patient underwent a laparotomy immediately for retroperitoneal exploration, which revealed more than 500 mL of feculent fluid collection in the above-mentioned locations. However, the intra-abdominal cavity was clear without any contamination. A gangrenous ruptured appendix was identified with its entire length embedded in the retroperitoneum, draining fecal content into it and forming an abscess. Appendectomy was done cautiously making sure that no necrotic residual appendix remained in the retroperitoneal cavity. The abscess was communicated with those at the thigh as expected, through the femoral canal just behind the inguinal ligament, even though a separate incision was made at the right thigh to ensure complete drainage of the abscess. Fortunately, the muscle groups of the right thigh were still viable and therefore no debridement of the muscle was performed. Multiple sump drainages were inserted at the end of the operation. The bacterial culture revealed an *Escherichia coli* infection.

The patient recovered smoothly with his fever subsiding 2 d after surgery and oral intake resumed in 4 d. Painful disability of his right thigh improved immediately after surgery and he was able to walk 12 d later. The patient was discharged uneventfully 3 wk after the surgery.

DISCUSSION

Acute appendicitis is the most common abdominal emergency worldwide and can usually be managed smoothly even if it is perforated. However, formation of retroperitoneal abscesses remains one of the most serious but rare complications of acute appendicitis and is always associated with perforation of a retrocecal appendix due to delayed diagnosis and treatment[5-7]. Knowing that the anatomical position of the appendix is variable and 65% of the appendix has been reported to be at the retrocecal[8], the importance of early management for acute appendicitis cannot be over-emphasized.

There are quite a few case reports discussing related problems similar to the present case, such as psoas and thigh abscesses[9,10], lower extremity subcutaneous

emphysema of abdominal origin[11-15] and rare complications of acute appendicitis[16,17]. Among these reports, descriptions are similar regarding patients' presentation and their management. In short, the onset of symptoms is usually insidious and atypical, initial medical treatment is usually unsuccessful. The causes of abscess formation are usually unclear before surgery and patients are usually critical on presentation. Surgical management is mandatory but may or may not be effective and mortality is not an uncommon result. In addition to the above-mentioned experiences, there are still some important issues that can be addressed based on our present case and a literature review.

First of all, it is still difficult to distinguish primary from secondary psoas abscesses. While the primary psoas abscess is defined as an abscess of unknown origin, its diagnostic symptoms and signs are almost identical to those of the secondary psoas abscess[9,10]. For example, the insidious onset of abscess formation is not responsive to medical treatment, the classical triads of psoas abscess such as fever, flank pain, and limitation of hip movement, can all present in both primary and secondary psoas abscesses[6-8,11]. After reviewing the information from the literature, we found that only the results of bacterial culture, if could be obtained before surgery, could indicate the nature of the abscess being primary or secondary. The most common causative pathogen of primary psoas abscess is *Staphylococcus aureus*, while that of secondary psoas abscess is usually mixed intestinal floras[9,10,12,18].

Second, as CT scan is used as a modality in addition to the physical signs for definite diagnosis in most of the reported cases, there is no doubt that CT scan of the abdomen with contrast is the most widely-used imaging study with the highest accuracy and efficiency[5-10,19]. CT scan of the abdomen not only helps in the establishment of the diagnosis, but also in the evaluation of the extension of involvement and in its treatment. For example, application of advanced high speed helical CT (which was the case in this report and has never been demonstrated before) can demonstrate a reconstructed coronal image that is of great help for surgical planning because it simulates the operation field. In addition, the drainage of abscess can be achieved by percutaneous and retroperitoneal approach or by laparotomy based on CT findings.

Third, whether the abscess is managed surgically or non-surgically should be carefully evaluated. With the improving technique and the accumulating experiences of interventional radiology, there are several reports demonstrating substantial results by percutaneous drainage of the abscess and then by surgery only if percutaneous drainage fails or is contraindicated. For example, Benoist *et al*[20] demonstrated that percutaneous drainage can drain 81% postoperative abdominal abscesses in patients without sepsis at presentation. Cantasdemir *et al*[21] showed that primary and secondary iliopsoas abscesses can be successfully treated with percutaneous drainage in patients without secondary abdominal pathology. Percutaneous drainage of the abscesses is less invasive. Gerzof *et al*[22] have recommended that percutaneous drainage should be done for most complex abscesses, but we are still uncertain if the percutaneous approach is adequate for our patient, because our patient was in a critical condition requiring prompt drainage of the abscess which cannot be achieved by percutaneous method and the infection could not be controlled if there was a persistent existing focus as previously reported by Ushiyama *et al*[15]. In addition, according to the results reported by Benoist *et al*[20] and Cantasdemir *et al*[21], a combined therapy (that is drainage in combination with surgery) might be contraindicated in our patient because he was septic and had a persistent existing intra-abdominal focus. Hence, it is our experience that surgery is advantageous over percutaneous drainage in patients under critical conditions such as perforated appendicitis, diverticulitis or malignancy[14].

Fourth, though abscesses inside the thigh is due to direct extension from retroperitoneum, it might be wise to create a separate incision at the thigh to drain the abscess rather than from the trunk. Draining thigh abscess by an incision at the thigh has two advantages. First, the abscess can be more easily and directly approached. Second, the viability of muscle and fascia of the thigh as well as the need for further debridement can be adequately evaluated. This is supported by the fact that some of thigh abscesses can be cured by drainage alone[6,7], while others with extensive myonecrosis require debridement or amputation[5,13].

In conclusion, formation of huge retroperitoneal abscesses with thigh involvement is a serious complication of perforated acute appendicitis. To improve the treatment outcome, patients with retroperitoneal infection should undergo CT scan in order to find the origin of the infection and to choose the best way to drain the abscess.

REFERENCES

1 **Blomqvist PG**, Andersson RE, Granath F, Lambe MP, Ekbom AR. Mortality after appendectomy in Sweden, 1987-1996. *Ann Surg* 2001; **233**: 455-460

2 **Hale DA**, Molloy M, Pearl RH, Schutt DC, Jaques DP. Appendectomy: a contemporary appraisal. *Ann Surg* 1997; **225**: 252-261

3 **Lally KP**, Cox CS, Andrassy RJ. Appendix. In: Townsend CM, Beauchamp RD, Evers BM, Mattox KL: Sabiston textbook of surgery. 17th ed. Philadelphia: Elsevier Saunders, 2004: 1381-1399

4 **Ellis H**, Natbanson LK. Appendix and appendectomy. In: Zinner MJ, Schwartz SI, Ellis H. Maingot's abdominal operations. 10th ed. Connecticut: Appleton & Lange, 1997: 1191-1227

5 **Edwards JD**, Eckhauser FE. Retroperitoneal perforation of the appendix presenting as subcutaneous emphysema of the thigh. *Dis Colon Rectum* 1986; **29**: 456-458

6 **Gutknecht DR**. Retroperitoneal abscess presenting as emphysema of the thigh. *J Clin Gastroenterol* 1997; **25**: 685-687

7 **Sharma SB**, Gupta V, Sharma SC. Acute appendicitis presenting as thigh abscess in a child: a case report. *Pediatr Surg Int* 2005; **21**: 298-300

8 **El-Masry NS**, Theodorou NA. Retroperitoneal perforation of the appendix presenting as right thigh abscess. *Int Surg* 2002; **87**: 61-64

9 **Agrawal SN**, Dwivedi AJ, Khan M. Primary psoas abscess. *Dig Dis Sci* 2002; **47**: 2103-2105

10 **Kleiner O**, Cohen Z, Barki Y, Mares AJ. Unusual presentation of psoas abscess in a child. *J Pediatr Surg* 2001; **36**: 1859-1860

11 **Nicell P**, Tabrisky J, Lindstrom R, Peter M. Thigh emphysema and hip pain secondary to gastrointestinal perforation. *Surgery*

1975; **78**: 555-559
12 **Rotstein OD**, Pruett TL, Simmons RL. Thigh abscess. An uncommon presentation of intraabdominal sepsis. *Am J Surg* 1986; **151**: 414-418
13 **Jager GJ**, Rijssen HV, Lamers JJ. Subcutaneous emphysema of the lower extremity of abdominal origin. *Gastrointest Radiol* 1990; **15**: 253-258
14 **Kobayashi H**, Sakurai Y, Shoji M, Nakamura Y, Suganuma M, Imazu H, Hasegawa S, Matsubara T, Ochiai M, Funabiki T. Psoas abscess and cellulitis of the right gluteal region resulting from carcinoma of the cecum. *J Gastroenterol* 2001; **36**: 623-628
15 **Ushiyama T**, Nakajima R, Maeda T, Kawasaki T, Matsusue Y. Perforated appendicitis causing thigh emphysema: a case report. *J Orthop Surg (Hong Kong)* 2005; **13**: 93-95
16 **Kao CT**, Tsai JD, Lee HC, Wang NL, Shih SL, Lin CC, Huang FY. Right perinephric abscess: a rare presentation of ruptured retrocecal appendicitis. *Pediatr Nephrol* 2002; **17**: 177-180
17 **Chang TN**, Tang L, Keller K, Harrison MR, Farmer DL, Albanese CT. Pylephlebitis, portal-mesenteric thrombosis, and multiple liver abscesses owing to perforated appendicitis. *J Pediatr Surg* 2001; **36**: E19
18 **Ricci MA**, Rose FB, Meyer KK. Pyogenic psoas abscess: worldwide variations in etiology. *World J Surg* 1986; **10**: 834-843
19 **Haaga JR**. Imaging intraabdominal abscesses and nonoperative drainage procedures. *World J Surg* 1990; **14**: 204-209
20 **Benoist S**, Panis Y, Pannegeon V, Soyer P, Watrin T, Boudiaf M, Valleur P. Can failure of percutaneous drainage of postoperative abdominal abscesses be predicted? *Am J Surg* 2002; **184**: 148-153
21 **Cantasdemir M**, Kara B, Cebi D, Selcuk ND, Numan F. Computed tomography-guided percutaneous catheter drainage of primary and secondary iliopsoas abscesses. *Clin Radiol* 2003; **58**: 811-815
22 **Gerzof SG**, Johnson WC, Robbins AH, Nabseth DC. Expanded criteria for percutaneous abscess drainage. *Arch Surg* 1985; **120**: 227-232

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CASE REPORT

Management of patients with stercoral perforation of the sigmoid colon: Report of five cases

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Abstract

To our knowledge, stercoral perforation of the colon is rarely seen with fewer than 90 cases reported in the literature till date. We explored the principles of management to prevent impending mortality in five patients with this condition. Five patients, two males and three females, whose median age was 64 years, had sustained stercoral perforation of the sigmoid colon. Chronic constipation was the common symptom among these patients. Three patients underwent a Hartmann's procedure and another two were treated with segmental colectomy with anastomosis and diverting colostomy. There was one surgical mortality and the other patients had an uneventful hospital stay. Timely intervention to prevent and/or treat any associated sepsis along with extensive peritoneal lavage and surgical intervention to remove diseased colonic tissue at the primary stercoral ulceration site coupled with aggressive therapy for peritonitis are key treatment modalities in salvaging patients presenting with stercoral perforation of the colon.

Key words: Stercoral perforation; Colon; Management

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INTRODUCTION

Berry[1] presented the first case of stercoral perforation of the colon to the Pathological Society of London in 1894, and it is a rare event with fewer than 90 cases reported in the literature through 2002[2]. Stercoral perforation of the colon is an aggravating condition following successive bowel wall ischemic necrosis by fecal mass[3], and dictates a rather poor clinical outcome in patients with a compromised general condition[4,5]. We reviewed the history of five patients of stercoral perforation of colon at our hospital, in order to elucidate the appropriate intraoperative procedures and obtain clinical results which will enhance the percentage of accurate preoperative diagnosis and clarify the most feasible perioperative management.

CASE REPORTS

During the years 2001-2005, 22 patients with free perforation of the colon were hospitalized at Chang Gung Memorial Hospital in Chiayi. The etiology of perforation was cancer in nine patients, ruptured diverticulitis in five, iatrogenic colon perforation related to colonoscopy procedure in two, one patient with traumatic colon perforation, and the other five patients were diagnosed as stercoral perforation which fit the diagnostic criterion proposed by Maurer *et al*[3].

These five patients formed the basis for this brief report (Tables 1 and 2). There were two men and three women aged between 4-84 years. All gave a long history of serious and chronic constipation except for a little girl. Four patients also had concomitant diseases that might have enhanced the development of chronic constipation such as diabetic enteropathy, hypothyroidism, and hemiparesis sequelae of stroke.

These five patients presented with sudden and severe abdominal pain. On physical examination, diffuse peritonitis was present in all the patients along with high fever and leukocytosis. Four patients were found to have sub-diaphragmatic free air on a standing chest roentgenogram, but only one showed localized extraluminal air on abdominal CT scanning. All patients underwent emergency laparotomy by a general surgeon with a preoperative diagnosis of hollow organ perforation. The colorectal surgeon was summoned during surgery to verify an intraoperative finding of colonic perforation. All perforation sites were found to be located at the

Table 1 Perioperative data on patients with stercoral perforation of the colon

Patient Number	Gender	Age	Interval (day)[1]	Case history	X-ray finding	Peritonitis	Localization	Perforation size (cm) and site	Fecaloma
A	F	4	1	Chronic constipation	Subdiaphragmatic free air	Generalized	Mid-sigmoid Colon	1×0.8 Anti-mesocolic	Within abdominal cavity
B	M	70	3	Chronic constipation, Cushing's syndrome	Subdiaphragmatic free air	Generalized	Rectosigmoid colon junction	5×3 Anti-mesocolic	Within the colon
C	F	84	2	Chronic constipation, D.M.	Subdiaphragmatic free air	Generalized	Sigmoid Colon	3×2, Anti-mesocolic	Protruding through perforation
D	F	79	1	Chronic constipation, stroke	Subdiaphragmatic free air	Lower abdomen	Mid-sigmoid Colon	2.5×1.5, Anti-mesocolic	Within the colon
E	M	64	2	Chronic constipation, D.M., hypothyroidism, gouty arthritis	Extraluminal free air	Generalized	Sigmoid Colon	4×2, Anti-mesocolic	Within the colon

[1] between symptom to operation.

Table 2 Perioperative data of patients with stercoral perforation of the colon

Patient number	Pathology	Ascites culture[1]	Colonoscopy[2]	Stercoral ulcer at proximal colon	Operation procedures	Peritoneal lavage[3]	Complication
A	Fecal peritonitis	*E. coli, Enterococcus faecalis, B. Fragilis*	No	Undetectable	Segmental colectomy+diverting enterostomy	Plenty	Nil
B	Purulent ascites	*Enterococcus faecalis, B. fragilis*	Yes (65, A-colon)	Four shallow stercoral ulceration diffusely	Hartmann's operation+rectal mucus fistula	Moderate	Mortality (overwhelming sepsis at post-op 21st d)
C	Fecal peritonitis	*E. coli*	Yes (50, proximal T-colon)	No ulceration	Hartmann's operation+rectal mucus fistula	Massive	Superficial wound infection
D	Purulent ascites	*E. coli, Kleb. pneumonia, B. thetaiotaomicron*	Yes (75, A-colon)	No ulceration	Segmental colectomy+diverting enterostomy	Massive	Superficial wound infection
E	Purulent ascites	*E. coli, Enterococcus faecium, Bacteroides sp.*	Yes (80, A-colon)	No ulceration	Hartmann's operation+rectal mucus fistula	Massive	Fascial dehiscence

[1]Heavy flora cultured as. [2](cm) distance at the most proximal to perforation and location. [3]Massive: >10 000 mL; moderate: <6 000 mL; plenty: 1 500 mL.

antimesenteric part of the sigmoid colon with perforations ranging in size from 2 to 5 cm. The perforation margins were well circumscribed with no local inflammation or any other chronic process. Three patients underwent a Hartmann's operative procedure with colostomy and rectal mucous fistula, another two patients were treated with segmental resection of the diseased colon with anastomosis and diverting enterostomy.

During the operation four patients, with the exception of the little girl**,** underwent colonoscopy to clarify the extent of the lesion of severe stercoral ulceration with impending colon perforation. Colon lavage was also performed as permitted by the proximity of the lesion**.**

Hemodynamic improvement and reversal of hypotensive status was noted immediately after colectomy was performed.

Intraperitoneal irrigation with massive amounts of warm normal saline solution and adequate placement of drainage tubes preceded the closure of the celiotomy incision**.**

All patients received broad-spectrum intravenous antibiotics and nutritional support for the duration of their hospital stay. Diverting enterostomy was closed in two patients 3 mo later. Hartmann's procedure was reversed in two patients after a minimum time of one and a half years. There were one surgical mortality due to overwhelming sepsis, morbidities due to superficial wound infection in two patients and fascial dehiscence in one patient, respectively.

DISCUSSION

Colon perforation is a rather uncommon event usually caused by malignancy, amoebic colitis, diverticular disease, spontaneous perforation, stercoral ulceration, steroid therapy, trauma, and ulcerative colitis[4]. The usual definition of stercoral perforation is "Perforation of the large bowel due to pressure necrosis from a fecal mass"[6], and represents a cause of colon perforation. Stercoral perforation of the colon was very rare for it was

first described in 1894 and less than 90 cases have been reported in the literature till date. We had five patients (22%) with this condition presenting as colonic perforation (22 patients) at our hospital, and it was said to be the largest series involving stercoral perforation of the colon in a single medical institute within a short-term interval.

Severe chronic constipation is considered to be the main causative factor in the development of stercoral perforation of the colon[4,6-8]. In our series, each patient had a long history of chronic constipation requiring medication for relief; the youngest female patient did not have congenital Hirshsprung's disease based on the pathologic examination of the colectomy specimen.

Long-standing constipation may enhance the formation of stone-hard fecalomas and maintain a persistent pressure over the bowel wall leading to pressure necrosis of the mucosa. Nevertheless, stercoral ulceration of colonic mucosa does not always occur among constipation cases, and not every stercoral ulceration results in colon perforation.

Multiple fecalomas can result in multiple stercoral ulcerations[6]. One of our five patients and 28% of the cases found in the literature showed this characteristic feature. There are several reasons why the perforation sites are located in the antimesenteric aspect and in the sigmoid colon: (1) Hypoperfusive status existing in the antimesenteric aspect other than the mesenteric border; (2) due to its more distal location and more solid consistency, fecalomas tend to form in more distal aspects of the large intestine such as the sigmoid colon; (3) being the most narrow region of the entire large intestine stool has difficulty passing through the sigmoid colon which increases the intraluminal pressure to the point where it can compress submucosal capillary vessels and reduce perfusion of the colonic wall; (4) prolonged localized pressure on the colon wall causing pressure ulcerations to appear[3,5,9]. In this series of patients perforation was only found in the anti-mesenteric margin of the sigmoid colon and not in other colonic site.

Treatment of intra-abdominal sepsis was achieved by massive saline irrigation and perforation control with the intention of decreasing the bacterial load in the abdominal cavity and deterring the development of overwhelming sepsis[10].

The single mortality in this series differed from the other four cases in the reduced amount of peritoneal lavage received due to intraoperative hypothermia.

Broad spectrum antibiotics were initiated in all the patients after hospital admission for polymicrobial peritoneal cavity contamination resulting from intestinal perforation[11].

In reviewing the literature, it was noted that intraoperative colonoscopy to inspect the remainder of the colon for stercoral ulceration was not undertaken in cases of stercoral perforation of the colon. To our knowledge, we are the first group to perform intraoperative colonoscopic examination.

The purpose of intraoperative colonoscopic examination was to ensure the adequacy of the colonic resection and rule out the presence of additional stercoral ulcerations that might lead to delayed colonic perforation.

Intraoperative colonoscopy was performed within 10 min after the colon was cleared of impacted stools.

Only one patient was found to have more than one stercoral ulceration of the entire colon. Colectomy was performed to remove all stercoral ulcerated lesions in this patient, unfortunately, due to an immuno-compromised state the patient expired after developing overwhelming sepsis.

Maurer *et al*[3] postulated that colonic dilation and the presence of multiple fecalomas may indicate such additional stercoral ulceration and carried the risk of a second perforation. We concur with this point of view based on the findings during intraoperative colonoscopy in our series and recommend removing pathologically altered or dilated colon segments to prevent another episode of colon stercoral perforation.

Mortality related to colon stercoral perforation was reported to be high[4,6,9]. Analysis of the reports in the literature revealed additionally that 28% of patients with stercoral perforation of the colon have multiple stercoral ulcers in the colon and that substantial mortality is encountered if only minor surgical procedures are employed as treatment such as simple exteriorization of the perforation site or closure of perforation hole with the addition of a diverting enterostomy[6,9].

Two review articles[6,9] and one series[3] with optimal clinical outcome in relation to the treatment of colonic stercoral perforation recommended that resection of the colon segment with an end colostomy and either mucous fistula or Hartmann's closure of the rectum would encounter comparatively low mortality. In our series, we took surgery of resection of colon with anastomosis and loop diverting colostomy in two patients (case 1 and case 4) with limited intraperitoneal septic condition and acceptable general condition. There were some advantages doing this procedure such as simple closure of colostomy someday. The other three patients were performed Hartmann's procedure with rectal mucous fistula.

In our series, we undertook surgical colonic resection with anastomosis and loop diverting colostomy in two patients (case 1 and case 4) with limited intraperitoneal sepsis and an acceptable general medical condition. The advantage of performing this procedure resides in the possibility of performing a simple closure of the colostomy in the future. The other three patients underwent Hartmann's procedure with rectal mucous fistula.

Stercoral perforation of the colon is not purely a surgical condition as it is usually complicated by a medical illness such as a superimposed immuno-compromised state which imparts a poor prognosis. Despite efforts to correct Cushing's syndrome, sufficient nutritional support and aggressive antibiotics therapy, it was not possible to prevent the systemic sepsis in case 2.

In conclusion, a favorable outcome in the treatment of stercoral perforation depends upon: (1) immediate treatment of any underlying sepsis; (2) removal of all stercoral ulcerated diseased colonic tissue; (3) extensive peritoneal lavage; (4) aggressive therapy to counteract colonic perforation peritonitis and (5) appropriate treatment of any co-morbid medical conditions. Prompt

institution of these measures will enhance survival of the patient and contribute to achieving a low mortality rate in stercoral perforation of the colon.

REFERENCES

1 **Berry J**. Dilatation and rupture of sigmoid flexure short report. *Brit Med J* 1894; **1**: 301
2 **Patel VG**, Kalakuntla V, Fortson JK, Weaver WL, Joel MD, Hammami A. Stercoral perforation of the sigmoid colon: report of a rare case and its possible association with nonsteroidal anti-inflammatory drugs. *Am Surg* 2002; **68**: 62-64
3 **Maurer CA**, Renzulli P, Mazzucchelli L, Egger B, Seiler CA, Büchler MW. Use of accurate diagnostic criteria may increase incidence of stercoral perforation of the colon. *Dis Colon Rectum* 2000; **43**: 991-998
4 **Gekas P**, Schuster MM. Stercoral perforation of the colon: case report and review of the literature. *Gastroenterology* 1981; **80**: 1054-1058
5 **Maull KI**, Kinning WK, Kay S. Stercoral ulceration. *Am Surg* 1982; **48**: 20-24
6 **Serpell JW**, Nicholls RJ. Stercoral perforation of the colon. *Br J Surg* 1990; **77**: 1325-1329
7 **Carter DC**, Kirkpatrick JR. Stercoral perforation of the sigmoid colon. *Br J Surg* 1973; **60**: 61-63
8 **Bauer JJ**, Weiss M, Dreiling DA. Stercoraceous perforation of the colon. *Surg Clin North Am* 1972; **52**: 1047-1053
9 **Guyton DP**, Evans D, Schreiber H. Stercoral perforation of the colon. Concepts of operative management. *Am Surg* 1985; **51**: 520-522
10 Mandell: Principles and Practice of Infectious Diseases. 5th ed. Churchill Livingstone Inc, 2000: 824-837
11 **Gorbach SL**, Nahas L, Lerner PI, Weinstein L. Studies of intestinal microflora. I. Effects of diet, age, and periodic sampling on numbers of fecal microorganisms in man. *Gastroenterology* 1967; **53**: 845-855

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Loas Angeles Converntion Center, California
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Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.

Genotypes: *gyr*A, *arg* 1, *c myc*, *c fos*, *etc.*

Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*

Biology: *Helicobacter pylori, H pylori, E coli, etc.*

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World Journal of Gastroenterology standard of quantities and units

Number	Nonstandard	Standard	Notice
1	4 days	4 d	In figures, tables and numerical narration
2	4 days	four days	In text narration
3	day	d	After Arabic numerals
4	Four d	Four days	At the beginning of a sentence
5	2 hours	2 h	After Arabic numerals
6	2 hs	2 h	After Arabic numerals
7	hr, hrs,	h	After Arabic numerals
8	10 seconds	10 s	After Arabic numerals
9	10 year	10 years	In text narration
10	Ten yr	Ten years	At the beginning of a sentence
11	0,1,2 years	0,1,2 yr	In figures and tables
12	0,1,2 year	0,1,2 yr	In figures and tables
13	4 weeks	4 wk	
14	Four wk	Four weeks	At the beginning of a sentence
15	2 months	2 mo	In figures and tables
16	Two mo	Two months	At the beginning of a sentence
17	10 minutes	10 min	
18	Ten min	Ten minutes	At the beginning of a sentence
19	50% (*V/V*)	500 mL/L	
20	50% (m/*V*)	500 g/L	
21	1 M	1 mol/L	
22	10 μM	10 μmol/L	
23	1N HCl	1 mol/L HCl	
24	1N H_2SO_4	0.5 mol/L H_2SO_4	
25	4rd edition	4^{th} edition	
26	15 year experience	15- year experience	
27	18.5 kDa	18.5 ku,18 500u or M_r18 500	
28	25 $g.kg^{-1}/d^{-1}$	25 g/(kg·d) or 25 g/kg per day	
29	6900	6 900	
30	1000 rpm	1 000 r/min	
31	sec	s	After Arabic numerals
32	1 pg $\cdot L^{-1}$	1 pg/L	
33	10 kilograms	10 kg	
34	13 000 rpm	13 000 *g*	High speed; *g* should be in italic and suitable conversion.
35	1000 *g*	1 000 r/min	Low speed. *g* cannot be used.
36	Gene bank	GenBank	International classified genetic materials collection bank
37	Ten L	Ten liters	At the beginning of a sentence
38	Ten mL	Ten milliliters	At the beginning of a sentence
39	umol	μmol	
40	30 sec	30 s	
41	1 g/dl	10 g/L	10-fold conversion
42	OD_{260}	A_{260}	"OD" has been abandoned.
43	One g/L	One microgram per liter	At the beginning of a sentence
44	*A*260 nm	A_{260} nm	*A* should be in italic.
	$^{b}P<0.05$	$^{a}P<0.05$	In Table, no note is needed if there is no significance instatistics: $^{a}P<0.05$, $^{b}P<0.01$ (no note if $P>0.05$). If ther is a second set of *P* value in the same table, $^{c}P<0.05$ and $^{d}P<0.01$ are used for a third set: $^{e}P<0.05$, $^{f}P<0.01$.
45	$*F=9.87$, $^{\S}F=25.9$, $^{\#}F=67.4$	$^{1}F=9.87$,$^{2}F=25.9$, $^{3}F=67.4$	Notices in or under a table
46	KM	km	kilometer
47	CM	cm	centimeter
48	MM	mm	millimeter
49	Kg, KG	kg	kilogram
50	Gm, gr	g	gram
51	nt	N	newton
52	l	L	liter
53	db	dB	decibel
54	rpm	r/min	rotation per minute
55	bq	Bq	becquerel, a unit symbol
56	amp	A	ampere
57	coul	C	coulomb
58	HZ	Hz	
59	w	W	watt
60	KPa	kPa	kilo-pascal
61	p	Pa	pascal
62	ev	EV	volt (electronic unit)
63	Jonle	J	joule
64	J/mmol	kJ/mol	kilojoule per mole
65	$10\times10\times10cm^3$	10 cm×10 cm×10 cm	
66	N·km	KN·m	moment
67	$x\pm s$	mean±SD	In figures, tables or text narration
68	Mean±SEM	mean±SE	In figures, tables or text narration
69	*im*	im	intramuscular injection
70	*iv*	iv	intravenous injection
71	Wang et al	Wang *et al.*	
72	EcoRI	*Eco*RI	*Eco* in italic and RI in positive. Restriction endonuclease has its prescript form of writing.
73	Ecoli	*E.coli*	Bacteria and other biologic terms have their specific expression.
74	Hp	*H pylori*	
75	Iga	*Iga*	writing form of genes
76	*igA*	IgA	writing form of proteins
77	-70 kDa	~70 ku	