Original Paper

Immune Thrombocytopenic Purpura in Patients with Hepatitis C Virus Infection

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ABSTRACT

Background/Aims: Immune thrombocytopenic purpura could occur as an extrahepatic manifestation of hepatitis C virus infection. The aim of this study was to clinically analyze hepatitis C virus-positive cases with immune thrombocytopenic purpura and to examine the relationship between hepatitis C virus and immune thrombocytopenic purpura.

Methodology: Eight hepatitis C virus-positive patients with immune thrombocytopenic purpura were compared with 67 cases with chronic hepatitis C without immune thrombocytopenic purpura. We examined various clinical and hematological parameters including platelet and platelet-associated immunoglobulin G.

Results: Two men and 6 women with hepatitis C virus infection (age 58.0±11.8) were diagnosed with immune thrombocytopenic purpura. Platelet counts ($x10^4/mm^3$) were significantly lower in these 8 patients (2.9±2.1) than in chronic hepatitis C patients without immune thrombocytopenic purpura (16.7±0.3) ($P<0.001$). Hepatitis C virus infection predated immune thrombocytopenic purpura in 6 patients and none of the patients with immune thrombocytopenic purpura was infected with hepatitis C virus after the diagnosis. Three of the 6 patients with chronic hepatitis C, which predated immune thrombocytopenic purpura, were treated with interferon and 2 developed immune thrombocytopenic purpura after the treatment. None of them eradicated hepatitis C virus by interferon.

Conclusions: The fact that hepatitis C virus infection predated immune thrombocytopenic purpura in 6 of 8 patients suggests that hepatitis C virus could potentially induce immune thrombocytopenic purpura and interferon itself might induce immune thrombocytopenic purpura.

KEY WORDS: HCV; ITP; Interferon; Complications

ABBREVIATIONS: Hepatitis C Virus (HCV); Immune Thrombocytopenic Purpura (ITP); Platelet-Associated Immunoglobulin G (PAIgG); Ethylenediaminetetraacetic Acid (EDTA); Standard Deviation (SD); Enzyme-Linked Immunosorbent Assay (ELISA); Interferon (IFN); Aspartate Aminotransferase (AST); Alanine Aminotransferase (ALT); Million Units (MU)

INTRODUCTION

Hepatitis C virus (HCV) does not only cause liver disease but extrahepatic disorders such as mixed essential cryoglobulinemia, glomerulonephritis, thyroiditis, Sjögren's syndrome, porphyria cutanea tarda, lichen planus, idiopathic pulmonary fibrosis, IgA deficiency, Mooren's corneal ulcers, Behçet's syndrome, polyarthritis, Guillain-Barré syndrome and Immune thrombocytopenic purpura (ITP) (1). Most of the cases with extrahepatic manifestations of HCV can be explained as an autoimmune disorder induced by HCV although the precise mechanism involved in this process has not been fully elucidated. The role of HCV in the occurrence of ITP has been reported (2), although HCV does not always appear to be the main etiological agent of ITP. The prevalence of HCV or anti-HCV positive in patients with ITP is reported to be 9-36% (2-5). We previously reported the importance of platelet-associated immunoglobulin G (PAIgG) in reducing the platelet count in some patients with HCV infection (6). Here we report the clinical and virological characteristics of patients with ITP complicated with hepatitis C.

METHODOLOGY

Between 1995 and 2002, 8 ambulatory Japanese patients were diagnosed with ITP who were also positive for HCV at the First Department of Internal Medicine, Gunma University Hospital and related hospitals, Gunma prefecture, Japan. All patients fulfilled the usual criteria for ITP 1) less than $100x10^9/L$ platelets at 2 different occasions (excluding ethylenediaminetetraacetic acid [EDTA]-related thrombocytopenia), 2) a normal or increased number of megakaryocytes in an otherwise normal bone marrow, and 3) exclusion of other causes of peripheral thrombocytopenia including disseminated intravascular coagulation, thrombotic thrombocytic purpura, hypersplenism, primary antiphospholipid syndrome, connective tissue disease, lymphoproliferative disorder, or drug toxicity and so on. All patients underwent bone marrow aspiration. Sixty-seven cases with chronic hepatitis C without ITP including 32 males and 35 females, mean age; 49.7±1.3 years were served as controls.

The results were expressed as mean±standard deviation (SD). Sex, age, laboratory data, treatment

Hepato-Gastroenterology 2005; 52:1197-1200

TABLE 1 Case Criteria

Case 1	2	3	4	5	6	7	8	
Age, sex	47,m	66,f	44,f	65,f	65,f	62,f	42, m	73,f
Liver disease*1	CH	CH	CH	CH	CH	CH	CH	CH
diagnosis age (y.o.)	39	49	35	46	62	55	37	61
treatment*2 (effectivity)	IFN (ineffective)	SNMC UDCA	IFN (ineffective), UDCA	UDCA	SNMC, UDCA	IFN (ineffective)	-	UDCA
HCV or ITP predated	HCV	HCV	HCV	HCV	unknown	HCV	HCV	unknown
Blood transfusion	+	+	-	-	unknown	+	+	-
ITP diagnosis age (y.o)	40	57	39	63	62	58	41	66
treatment*3	none	predn	none	predn, CPM, splenectomy	predn	none	none	predn
effectivity	-	good	-	good	poor	-	-	poor
HCV genotype or serotype*4	1b	group 1	group 1	group 1	group 1	1b	1b	1b
Virus load (KIU/mL)	850<	764	220	320	447	770	850<	560
Platelet count (x10⁴/mL) first, lowest, recent	9.0-1.9-2.1	12.2-0.7-11.6	17.4-2.8-5.9	1.8-0.8-7.6	5.7-2.5-9.2	7.1-5.4-12.5	10.4-6.8-10.6	3.0-2.6-4.5
PAIgG (ng/10⁷ cells)	397.9	2037.2	198.7	637.7	210.2	386.7	419.4	326.8
AST (IU/L)	45	51	38	23	54	86	47	30
ALT (IU/L)	42	57	38	16	43	73	69	22
WBC (/mm³)	5600	4600	6500	2800	2300	4800	3700	5400
Mgk in bone marrow*5	++	++	++	++	++	++	+	++
Bleeding tendency	+	+	-	+	+	+	-	+
Splenomegaly	-	-	-	-	-	-	-	-
Complication	-	gall stone	-	- gall stone	hypertension, thyroiditis hypertension	chronic,		hypertension

*1: CH: chronic hepatitis; *2: IFN: interferon, SNMC: strong neominophagen C, UDCA: ursodeoxycholic acid; *3: predn: prednisolone, CPM: cyclophosphamide; *4: serotype resulted in 1 or 2. Serotype 1 includes genotype 1a and 1b; *5: +: normal, ++: megakaryocytosis.

and complications were analyzed in each patient. Anti-HCV antibody was used by the third-generation enzyme-linked immunosorbent assay (ELISA) method and HCV serotype was analyzed by solid-phase ELISA (7). HCV-RNA was detected by reverse transcriptase-polymerase chain reaction and PAIgG was determined by using a competitive micro-ELISA (6).

RESULTS

The patients included 2 men and 6 women with a mean age of 58.0 11.8 years (range, 42 to 73 years). Eight patients had chronic hepatitis. With regard to the onset of ITP in relation to HCV infection, HCV infection predated ITP in 6 patients and which predated could not be determined in the remaining 2. Four of 8 patients received blood transfusion and 3 patients did not receive one and no information was available in the remaining 1 patient. ITP was diagnosed at 53.3±11.3 years of age, 5.25 years after the detection of HCV **(Table 1)**.

Three patients were treated with interferon (IFN) but none showed clearance of HCV after the therapy. Two developed ITP after the therapy. Thus, ITP seemed to be induced by IFN in 2 of the 3 patients who received such treatment. On the other hand, 4 patients received steroid therapy, which was effective in 2 of these patients and resulted in clinical improvement and increase in platelet count. Splenectomy was performed in 1 patient and was effective in improvement of thrombocytopenia.

All patients with HCV complicated by ITP had serotype 1 of anti-HCV or genotype 1b. The mean serum HCV-RNA level was 598±247 KIU/mL in ITP group and the level was more than 100 KIU/mL in each patient. The average of lowest platelet count (x10⁴ /mm³) recorded in these 8 patients (2.94±2.14) was significantly lower than in 67 patients with chronic hepatitis C but without ITP (16.7±0.3) (P<0.001). The mean titer of PAIgG (ng/platelet 10⁷ cells) was not significantly higher in ITP group (576.8±605.8) than in non-ITP group (87.3±10.1) (P=0.056). Serum transaminase was <100 IU/L in all patients, with a mean aspartate aminotransferase (AST)/alanine aminotransferase (ALT) value of 46.8/45.0, and the maximum value was 86/73. Leukocyte count ranged from 2300 to 6500/mm³ (average, 4463±1438/mm³). Bone marrow aspiration demonstrated megakaryocytosis in 7 of 8 patients. Bleeding tendency was seen in 6 cases. Splenomegaly was not observed in all cases.

Case Reports

We report here 2 cases that developed ITP after IFN therapy:

Case 1 was treated with 9 million units (MU) of recombinant IFN- 2a daily for 4 weeks, followed by three times a week. Platelet counts were 7.5x10⁴ /mm³ before IFN therapy but decreased to 1.8x10⁴ /mm³ at the end of the 8 weeks of IFN therapy. IFN was stopped and platelet count promptly recovered to 6.8x10⁴ /mm³. IFN was re-administered but resulted

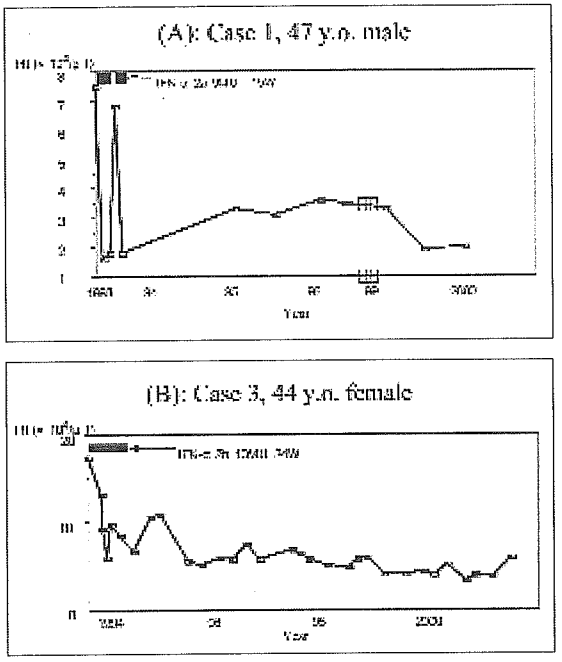

FIGURE 1 Two cases who developed ITP after IFN therapy. **(A)** Case 1. 47-year-old male. **(B)** Case 3. 44- year-old female.

in a decrease in platelet count to 1.8x10^4 /mm^3, necessitating withdrawal of IFN therapy. However, platelet count did not recover remains at present at around 1 to 2x10^4 /mm^3, 10 years after therapy **(Figure 1A)**.

Case 3 received 10 MU of recombinant IFN- 2b daily for 4 weeks, followed by 3 times weekly for 20 weeks. Platelet counts were 17.4x10^4 /mm^3 before IFN therapy but decreased to 13.2x10^4 /mm^3 2 weeks after the start of the therapy and further fell to 8.4x10^4 /mm^3 at the end of IFN therapy. After the completion of IFN therapy, platelet count did not recover to the pre-treatment level and is currently about 3 to 5x10^4 /mm^3, 9 years after therapy **(Figure 1B)**.

DISCUSSION

We identified 8 patients with ITP related to HCV infection among more than 500 HCV-positive patients in Gunma prefecture, Japan. The present data reflect an ITP incidence of 1.6% at most among HCV-positive patients. The incidence was far from high. But HCV infection clearly predated ITP in 6 of HCV positive patients with ITP. Pawlotsky *et al.* (2) expressed that patients with ITP could be at risk of HCV transmission by blood products used in the treatment of ITP, but in this study there was no patient that ITP predated HCV infection. Therefore HCV might potentially induce ITP in low incidence.

All cases reported in our study were of group 1 or genotype 1b of HCV serotype or genotype and had a HCV viral load of >100 KIU/mL. The above features might be characteristic for HCV-related ITP. All cases were free of splenomegaly.

ITP is an autoimmune disease characterized by increased platelet clearance caused by antiplatelet autoantibodies. Autoantibodies to platelet surface receptors such as glycoproteins IIb/IIIa are thought to cause their phagocytosis by monocytes and macrophages in the spleen and liver (8,9). There are no data regarding the relationship between the epitope of platelet surface glycoprotein and HCV. We previously reported the relationship between HCV and thrombocytopenia (6). Patients with HCV infection tended to have low platelet counts and high titers of PAIgG, compared with hepatitis B. Furthermore, treatment with IFN to eradicate HCV resulted in a gradual increase of platelet count and decrease of PAIgG (data not shown). Based on our limited experience, it seems that IFN is not appropriate for treatment of patients with chronic hepatitis C complicated with ITP, because of its side effects especially thrombocytopenia. In this regard, there are some reports of severe immune thrombocytopenia during -IFN therapy for chronic viral hepatitis. A French study reported that IFN- could have potentially deleterious effects in HCV-associated ITP (10). Dourakis *et al.* also reported two patients who developed -IFN-induced thrombocytopenia, with features of autoimmune thrombocytopenic purpura (11). Fujii *et al.* also reported a similar life-threatening case of severe immune thrombocytopenia (12). In addition, IFN can also itself induce idiopathic thrombocytopenic purpura, as indicated previously (13).

Drug-induced immune thrombocytopenia generally shows spontaneous recovery soon after the cessation of such therapy. However, the two cases presented in this study showed persistently low platelet counts after cessation of IFN therapy. IFN is known to suppress megakaryocyte production. Because megakaryocytosis in bone marrow were recognized in these cases, this fact suggests IFN acted as peripheral destruction of platelets, not as suppression of megakaryocytes production. Such treatment seemed to affect not only platelets but also the entire immune system. Immunological disturbances induced by IFN might be associated with permanent or persistent long-term thrombocytopenia.

On the other hand, IFN- has been reported to improve ITP (14-17) but not all cases respond to IFN- (18). These results emphasize the challenge facing physicians when treating patients with ITP based on the potential side effects of IFN, namely thrombocytopenia. In fact, in our series of ITP patients with HCV infection, treatment with IFN did not result in any beneficial effects. These contradicting data point to the need of further studies of large population samples to examine this issue more carefully in order to provide guidelines for treatment of patients with HCV complicated with ITP.

REFERENCES

1 **Hadziyannis SJ:** The spectrum of extrahepatic manifestations in hepatitis C virus infection. J Viral Hepatitis 1997; 4:9-28.

2 **Pawlotsky JM, Bouvier M, Fromont P, et al:** Hepatitis

C virus infection and autoimmune thrombocytopenic purpura. J Hepatol 1995; 23:635-639.
3 **Pivetti S, Novarino A, Merico F, et al:** High prevalence of autoimmune phenomena in hepatitis C virus antibody positive patients with lymphoproliferative and connective tissue disorders. Br J Haematol 1996; 95:204-211.
4 **Sakuraya M, Murakami H, Uchiumi H, et al:** Steroid-refractory chronic idiopathic thrombocytopenic purpura associated with hepatitis C virus infection. Eur J Haematol 2002; 68:49-53.
5 **Bauduer F, Marty F, Larrouy M, Ducout L:** Immunologic thrombocytopenic purpura as presenting symptom of hepatitis C infection. Am J Hematol 1998; 57:338-340.
6 **Nagamine T, Ohtuka T, Takehara K, Arai T, Takagi H, Mori M:** Thrombocytopenia associated with hepatitis C viral infection. J Hepatol 1996; 24:135-140.
7 **Tanaka T, Tsukiyama-Kohara K, Yamaguchi K, et al:** Significance of specific antibody assay for genotyping of hepatitis C virus. Hepatology 1994; 19:1347-1353.
8 **Kuwana M, Kaburaki J, Kitasato H, et al:** Immunodominant epitopes on glycoprotein IIb-IIIa recognized by autoreactive T cells in patients with immune thrombocytopenic purpura. Blood 2001; 98:130-139.
9 **Kuwana M, Okazaki Y, Kaburaki J, Kawakami Y, Ikeda Y:** Spleen is a primary site for activation of platelet-reactive T and B cells in patients with immune thrombocytopenic purpura. J Immunol 2002; 168:3675-3682.
10 **Bacq Y, Sapey T, Gruel Y, et al:** Exacerbation of an autoimmune thrombocytopenic purpura during treatment with interferon alpha in a woman with chronic viral hepatitis C. Gastroenterol Clin Biol 1996; 20:303-306.
11 **Dourakis SP, Deutsch M, Hadziyannis SJ:** Immune thrombocytopenia and -interferon therapy. J Hepatol 1996; 25:972-975.
12 **Fujii H, Kitada T, Yamada T, Sakaguchi H, Seki S, Hino M:** Life-threatening severe immune thrombocytopenia during Alfha-interferon therapy for chronic hepatitis C. Hepatogastroenterology 2003; 50:841-842.
13 **Murakami CS, Zeller K, Bodenheimer HC, Jr., Lee WM:** Idiopathic thrombocytopenic purpura during interferon- 2B treatment for chronic hepatitis. Am J Gastroenterol 1994; 89(12):2244-2245.
14 **Durand JM, Cretel E, Retornaz F, Lefevre P, Kaplanski G, Soubeyrand J:** Alpha interferon therapy in thrombocytopenia associated with hepatitis C virus infection. J Hepatol 1994; 21:277-279.
15 **Yesilipek MA, Yegin O:** Interferon-alpha therapy for refractory idiopathic thrombocytopenic purpura in children. Turk J Pediatr 1997; 39(2):173-176.
16 **Secreta CM, Baker DE:** Interferon alfa therapy in adults with chronic idiopathic thrombocytopenic purpura. Ann Pharmacother 1996; 30(10):1176-1179.
17 **Mizukoshi E, Tanaka N, Fukuoka K, Toya T:** A case of successful interferon therapy for chronic type C hepatitis associated with idiopathic thrombocytopenic purpura. Acta Hepatol Jap 1994; 35(8):617-622. (In Japanese with English abstract)
18 **Vianelli N, Tazzari PL, Baravelli S, Ricci F, Valdre L, Tura S:** Interferon-alpha 2b is not effective in the treatment of refractory immune thrombocytopenic purpura. Haematologica 1998; 83(8):761-763

Liver International 2005: 25: 28–32
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Liver International
DOI: 10.1111/j.1478-3231.2004.0986.x

Clinical Studies

Elevated plasma adiponectin concentrations in patients with liver cirrhosis correlate with plasma insulin levels

Sohara N, Takagi H, Kakizaki S, Sato K, Mori M. Elevated plasma adiponectin concentrations in patients with liver cirrhosis correlate with plasma insulin levels.
Liver International 2005: 25: 28–32. © Blackwell Munksgaard 2004

Abstract: *Background:* Adiponectin is a hormone secreted by adipocytes and has anti-diabetic and anti-atherogenic properties. Hypoadiponectinemia is associated with insulin-resistant diabetes and liver dysfunction. The aim of this study was to determine plasma adiponectin and insulin levels in patients with liver cirrhosis. *Methods:* Adiponectin and insulin levels were determined in 38 patients with cirrhosis and 30 healthy controls, and were correlated with various clinical and biochemical parameters. Patients included 21 with Child A, eight Child B, and nine with Child C liver cirrhosis. *Results:* Log adiponectin and insulin levels were significantly elevated in patients with cirrhosis compared with the control. In liver cirrhosis, the level of adiponectin increased proportionately with the Child's classification score. In control subjects, plasma adiponectin correlated inversely with insulin levels. In contrast, plasma adiponectin correlated positively with insulin levels in patients with liver cirrhosis. Plasma adiponectin levels did not correlate with age, sex, body mass index, total bilirubin, aspartate aminotransferase, and fasting blood sugar levels in both groups, while alanine aminotransferase correlated negatively with adiponectin in control subjects as reported previously. *Conclusion:* Our results of high plasma adiponectin in patients with liver cirrhosis could reflect an imbalance between its production by adipocytes and metabolism in the liver.

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Key words: adiponectin – insulin – liver cirrhosis

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Received 23 April 2004,
accepted 19 July 2004

Adiponectin or Acrp30 is an adipose tissue-specific protein (1–3) and one of the most abundant gene transcript proteins in adipocytes, corresponding to ~0.01% of all proteins (4). The biological activities of adiponectin are poorly understood. However, previous studies reported the detection of low adiponectin concentrations in insulin-resistant patients with obesity, type 2 diabetes mellitus, or coronary artery disease (5–7). Injection of recombinant intact or fragment adiponectin reduced blood glucose, overcame insulin resistance, decreased fatty acid, and caused weight loss in obese mice (8–10). An inverse relationship between insulin resistance and plasma adiponectin levels was also reported in adiponectin knockout mice (11). It is understood that metabolic changes in muscles and hepatocytes seem to account for these systemic changes.

In chronic liver disease (CLD) such as liver cirrhosis, impaired insulin sensitivity and subsequent alterations in glucose metabolism, such as high prevalence of insulin resistance and glucose intolerance, are reported (12). Nearly all patients with liver cirrhosis are insulin resistant, 60–80% are glucose intolerant, and about 20% develop clinical manifestations of diabetes mellitus (12). In patients with liver cirrhosis, it was reported that chronic hyperinsulinemia causes insulin resistance (13). However, the etiological mechanism of impaired insulin-mediated glucose utilization remains unknown. Another report showed that serum adiponectin levels negatively correlated with serum transaminase levels (14).

The above background suggests a close link among insulin resistance, liver function, and circulating adiponectin levels. However, there is little information regarding adiponectin regulation in liver cirrhosis. The present study was designed to define the relationship between circulating adiponectin levels and liver cirrhosis.

Research design and methods

The subjects were CLD inpatients evaluated for treatment at Gunma University Hospital and age-matched normal control volunteers. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, was approved by the local ethical committee, and informed consent was obtained from each participant. All blood samples were obtained in the morning after an overnight fast. Table 1 summarizes the clinical background of 30 control subjects and 38 CLD patients who were enrolled in this study. Liver cirrhosis was diagnosed based on clinical features, laboratory tests, and computed tomographic findings. Plasma adiponectin concentrations were determined by enzyme-linked immunosorbent assay (ELISA) (adiponectin ELISA kit, Otsuka Pharmaceutical Co, Tokyo, Japan). Total bilirubin (T-Bil), aspartate aminotransferase (AST), alanine aminotransferase (ALT), fasting blood sugar (FBS), and fasting blood insulin were measured using standard techniques. Body mass index (BMI) was calculated as weight divided by the square of height in meters.

Statistical analysis

Data were expressed as mean ± SD. Differences between groups were analyzed by the Mann–Whitney's *U*-test, and multiple comparisons used Kruskal–Wallis analysis of variances (ANOVA). Relations between variables were sought by Spearman's rank correlation coefficients and by linear regression analysis with forward selection. Comparisons between subgroups are illustrated with box-plot graphics, where the dotted line within the box indicates the median value, and the box boundaries represent 50% of the values of non-outliners. The threshold for significance was set at $P<0.05$.

Table 1. Clinical characteristics of the patients with chronic liver disease

Characteristic	Control ($n=30$)	Chronic liver disease (n = 38)	*P*-value
Sex (male:female)	19:11	26:12	NS
Age (years)	63.0 ± 15.5	66.8 ± 8.5	NS
Body weight (kg)	57.4 ± 12.2	55.3 ± 10.4	NS
BMI (kg/m^2)	22.5 ± 3.6	21.9 ± 2.7	NS
Child A:Child B:Child C	–	21:08:09	
T-Bil (mg/dl)	0.81 ± 0.20	1.78 ± 1.40	<0.05
AST (IU/l)	24.9 ± 6.9	51.1 ± 22.1	<0.05
ALT (IU/l)	27.3 ± 10.0	40.7 ± 28.3	<0.05
Fasting plasma glucose (mg/dl)	100.7 ± 18.5	138.6 ± 90.6	<0.05
Fasting plasma insulin (mg/dl)	4.6 ± 2.3	15.7 ± 18.1	<0.05
Loe adioonectin (μg/ml)	0.35 ± 0.10	0.46 ± 0.12	<0.05

Data represent mean ± SD. NS, not significant; BMI, body mass index; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; SD, standard deviation.

Results

Clinical characteristics and plasma adiponectin levels in CLD patients and controls

This study comprised 68 subjects, including 30 control subjects (19 males/11 females, mean age, 63.0 – 15.5 years) and 38 patients with liver cirrhosis (26 males/12 females, 66.8 – 8.5 years). The diagnosis was liver cirrhosis in all patients. None of the patients received blood transfusion or was on high-dose steroids at the time of the study. The severity of liver cirrhosis, defined by Child's classification, in our patients is summarized in Table 1. The underlying causes of liver disease were alcohol ($n=1$), hepatitis B ($n=3$), hepatitis C ($n=31$), and unknown origin ($n=3$). Patients with liver cirrhosis were similar to the controls with regard to sex, age, body weight, and BMI. Serum T-Bil, AST, and ALT were significantly higher in cirrhotic patients than the controls. FBS of 16/38 cirrhotic patients and 1/30 controls were above 126 mg/dl, and the mean FBS value of cirrhotic patients was significantly higher than that of the control. Log adiponectin and insulin levels were significantly elevated in patients with liver cirrhosis compared with the control ($P<0.05$).

Plasma adiponectin levels and liver damage

Plasma adiponectin levels were significantly elevated in patients with Child A ($n=21$, $P<0.05$) and Child B and C ($n=17$, $P<0.05$) liver cirrhosis compared with controls ($n=30$). Furthermore, plasma adiponectin levels of patients with Child A liver cirrhosis were of intermediate level between the control and patients with Child B and C liver cirrhosis (Fig. 1a). Similar results were noted for fasting plasma insulin levels; insulin increased progressively with worsening of liver cirrhosis defined by Child's classification (Fig. 1b).

Correlation between adiponectin and clinical data

In the control subjects, plasma adiponectin concentrations correlated inversely with serum ALT ($r=-0.511$, $P<0.01$) and plasma insulin ($r=-0.404$, $P<0.05$, Spearman's rank correlation, Table 2). However, the opposite correlation was observed in patients with liver cirrhosis. Thus, adiponectin correlated positively and sig-

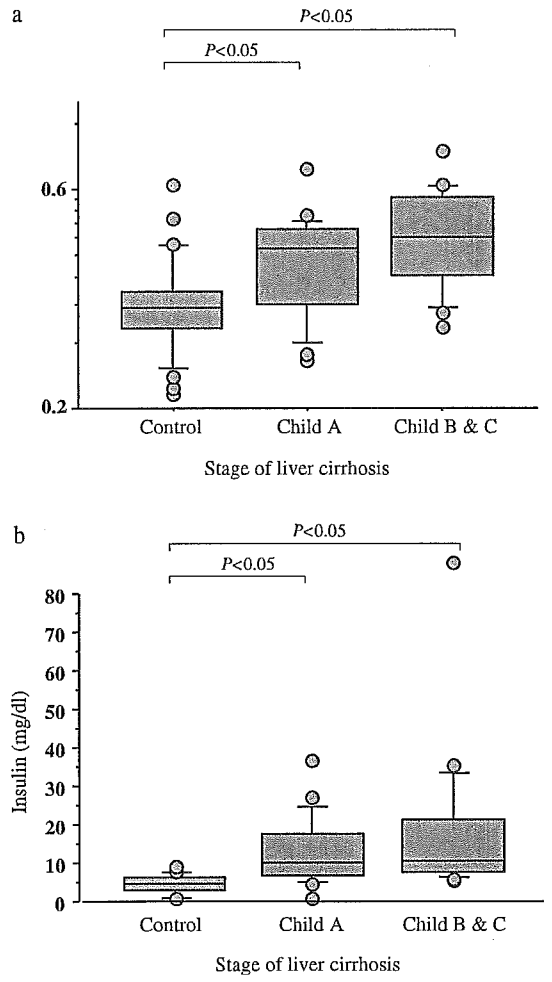

Fig. 1. Plasma adiponectin and insulin levels in liver cirrhosis. (a) Adiponectin is elevated in Child A (0.43–0.11, $P<0.05$) and Child B and C (0.50–0.12, $P<0.05$) cirrhosis compared with the control (0.35–0.10). (b) Insulin is elevated in Child A (12.6–8.8, $P<0.05$) and Child B and C (19.5–25.1, $P<0.05$) cirrhosis compared with the control (4.6–2.3) (data are mean ± SD).

nificantly with insulin ($r = 0.462$, $P<0.01$, Spearman's rank correlation, Table 2). We did not find any correlation between plasma adiponectin levels and age, BMI, T-Bil, AST, and FBS in both groups, and AST in the liver cirrhosis group.

The regression equation indicated a negative correlation between adiponectin and insulin in control subjects ($r = -0.429$, $P = 0.017$, $R^2 = 0.184$, Fig. 2a) and a positive correlation in patients with liver cirrhosis ($r = 0.354$, $P = 0.028$, $R^2 = 0.124$, Fig. 2b)

Table 2. Association between adiponectin and measures of characteristics

	Controls		Chronic liver disease	
	r-value	*P*-value	*r*-value	*P*-value
Sex	0.113	NS	0.132	NS
Age	0.28	NS	0.155	NS
BMI	0.346	NS	−0.144	NS
T-Bil	0.113	NS	0.199	NS
AST	−0.311	NS	0.07	NS
ALT	−0.511	<0.01	0.303	NS
Fasting plasma glucose	−0.349	NS	−0.006	NS
Fasting plasma insulin	−0.357	<0.05	0.462	<0.01

Spearman's rank correlation. NS, not significant; BMI, body mass index; T-Bil, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

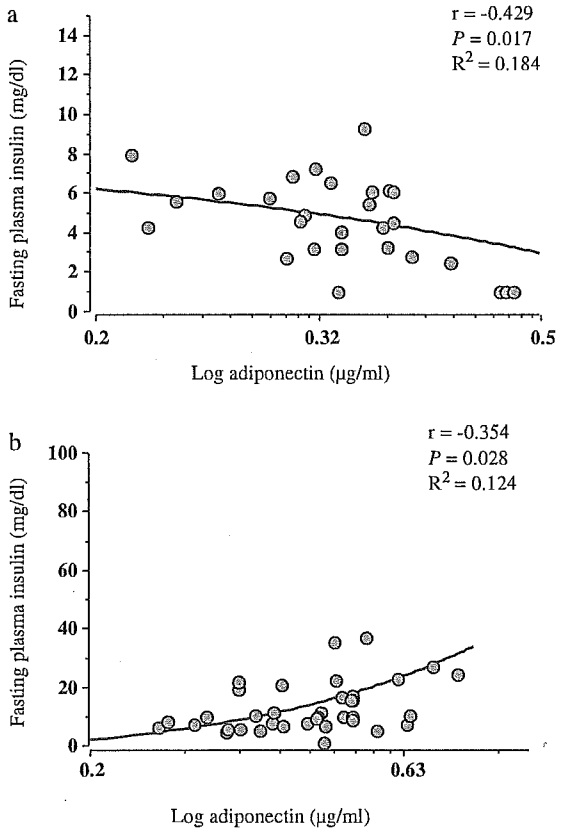

Fig. 2. Correlation between adiponectin and insulin. (a) In control subjects, plasma adiponectin concentrations are inversely correlated with insulin concentrations ($P<0.05$). (b) In patients with liver cirrhosis, plasma adiponectin concentrations are positively correlated with insulin concentrations ($P<0.01$).

Discussion

The major findings of the present study were as follows: (1) the presence of high plasma adiponectin levels in patients with liver cirrhosis relative to the control, (2) these levels increased in proportion with the severity of liver cirrhosis, and (3) the negative correlation between plasma adiponectin and insulin levels in control subjects was reversed in patients with liver cirrhosis.

Adiponectin has attracted considerable attention as a hormone secreted by adipocytes that can

regulate glucose levels in the circulation, overcome insulin resistance, and cause weight loss. It is also reported that insulin-resistant individuals with obesity, type 2 diabetes mellitus, or coronary artery disease have low adiponectin concentrations (5–7). The plasma level of adiponectin is proposed to be influenced by nutritional status, because it is reported that adiponectin is decreased in obesity (15), and weight reduction causes the plasma adiponectin level to be increased (16). Moreover, serum total cholesterol and triglyceride levels have negative correlations with adiponectin in human (15). From this viewpoint, we hypothesized that plasma adiponectin levels in patients with liver cirrhosis would be increased because they usually tend to be malnourished (17).

In our study, circulating adiponectin concentration was significantly increased in patients with liver cirrhosis compared with the control. Furthermore, plasma adiponectin concentrations increased in a stepwise fashion with higher grades of Child's classification (Fig. 1a), although the difference in the concentration between Child A and Child B and C groups did not reach statistical significance, probably because of the small sample numbers.

A number of recent studies reported low adiponectin production in obese subjects, and that reduced secretion of adiponectin from visceral adipose tissue accounted for the low plasma adiponectin concentrations observed in obesity (15). Furthermore, an *in vitro* study showed that cultured visceral adipocytes secreted adiponectin more actively than subcutaneous adipocytes (18), and another study reported that adiponectin secretion from visceral adipocytes correlated negatively with BMI of participating subjects (19). In our study, the BMI values of the control subjects and patients with liver cirrhosis were almost similar. To our knowledge, there are no established studies that showed the difference in the distribution of visceral and subcutaneous adipose tissues between normal subjects and patients with liver cirrhosis. Increased plasma levels of adiponectin in liver cirrhosis could be because of either increased production or reduced metabolism. At present, it is difficult to say that there is a large difference in adiponectin production between patients with liver cirrhosis and control subjects, as the BMI values of both groups are almost same in our study. On the other hand, the metabolism of adiponectin in such patients is still unknown. It is therefore possible that the stepwise increase in plasma adiponectin levels with the progression of Child's score is based on the liver being one of the main organs involved in adiponectin metabolism.

Lopez-Bermejo et al. (14) reported that adiponectin correlated negatively with ALT and immunoreactive insulin levels. Our study showed similar data in control subjects; ALT and fasting insulin levels correlated negatively with serum adiponectin levels in healthy subjects. However, in patients with liver cirrhosis, our results showed a reversal of the correlation between adiponectin and insulin together with elevation of serum adiponectin levels. With regard to serum adiponectin levels and liver function, Lopez-Bermejo et al. (14) investigated subjects with normal liver function, including normal controls and/or patients with chronic hepatitis, but not patients with liver cirrhosis. We examined blood samples from patients with liver cirrhosis who showed deterioration of liver function and insulin resistance. Plasma adiponectin and insulin levels were elevated and correlated with the progression of Child's classification. Patients with liver cirrhosis commonly display chronically elevated plasma insulin concentrations that result from a complex relationship between the ability of β cells to compensate for the insulin-resistant state, the degree of impaired hepatic insulin degradation, and the extent of portal hypertension (12). It is still unclear why adiponectin and insulin changes show a similar pattern both in control subjects and patients with liver cirrhosis. However, it appears certain to us that the liver metabolism function for adiponectin and insulin would largely contribute to these changes. Further studies are necessary to figure out these relationships.

Similar results have just appeared elsewhere (20). Tietge et al. demonstrated that the liver was the major source of adiponectin extraction and adiponectin plasma levels in cirrhosis are significantly elevated compared with normal, because of reduced liver function and hepatic hemodynamics. But the positive correlation of insulin and adiponectin in patients with liver cirrhosis in our paper was not obtained in their paper possibly because of the small number of the cases.

In conclusion, in the present study we have demonstrated the presence of higher plasma adiponectin concentrations in patients with liver cirrhosis compared with the control, and that the negative correlation between adiponectin and insulin in control subjects was reversed in patients with liver cirrhosis.

Acknowledgements

The authors thank Prof. M. Murakami (Gunma University Graduate School of Medicine) for his assistance with this study.

References

1. Hu E, Liang P, Spiegelman B M. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 1996; 271: 10697–703.
2. Gerg A H, Combs T P, Scherer P E. An adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 2002; 13: 84–9.
3. Yoda-Murakami M, Taniguchi M, Takahashi K, et al. Change in expression of GBP28/adiponectin in carbon tetrachloride-administrated mouse liver. Biochem Biophys Res Commun 2001; 285: 372–7.
4. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (adipose most abundant gene transcript 1). Biochem Biophys Res Commun 1996; 221: 286–9.
5. Ohchi N, Kihara S, Arita Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999; 100: 2473–6.
6. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999; 257: 79–83.
7. Hotta K, Funahashi T, Arita Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000; 20: 1595–9.
8. Yamauchi T, Kamon J, Waki H, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001; 7: 941–6.
9. Berg A H, Combs T P, Du X, Brownlee M, Scherer P E. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001; 7: 947–53.
10. Fruebis J, Tsao T S, Javorschi S, et al. Proteolytic cleavage production of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and cause weight loss in mice. Proc Natl Acad Sci USA 2001; 98: 2005–10.
11. Maeda N, Shimomura I, Kishida K, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 2002; 8: 731–7.
12. Petrides A S. Liver disease and diabetes mellitus. Diabetes Rev 1994; 2: 2–18.
13. Petrides A S, Stanley T, Matthews D E, Vogt C, Bush A J, Lambeth H. Insulin resistance in cirrhosis: prolonged reduction of hyperinsulinemia normalizes insulin sensitivity. Hepatology 1998; 28: 141–9.
14. Lopez-Bermejo A, Botas P, Funahashi T, et al. Adiponectin, hepatocellular dysfunction and insulin sensitivity. Clin Endocrinol (Oxf) 2004; 60: 256–63.
15. Havel P J. Control of energy homeostasis and insulin action by adipocyte hormone: leptin, acylation stimulating protein, and adiponectin. Curr Opin Lipidol 2002; 13: 51–9.
16. Yang W S, Lee W J, Funahashi T, et al. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab 2001; 86: 3815–9.
17. Lautz H U, Selberg O, Korber J, Burger M, Muller M J. Protein-calorie malnutrition in liver cirrhosis. Clin Invest 1992; 70: 478–86.
18. Park K G, Park K S, Kim M J, et al. Relationship between serum adiponectin and leptin concentrations and body fat distribution. Diabetes Res Clin Pract 2004; 63: 135–42.
19. Motoshima H, Wu X, Shnha M K, et al. Differential regulation of adiponectin secretion from cultured human omental and subcutaneous adipocytes: effects of insulin and rosiglitazone. J Clin Endocrinol Metab 2002; 87: 5662–7.
20. Tietge U J, Boker K H, Manns M P, Bahr M J. Elevated circulating adiponectin levels in liver cirrhosis are associated with reduced liver function and altered hepatic hemodynamics. Am J Physiol Endocrinol Metab 2004; 287: E82–9.

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World J Gastroenterol 2005;11(47):7444-7449
World Journal of Gastroenterology ISSN 1007-9327

• *BASIC RESEARCH* •

Overexpression of NK2 inhibits liver regeneration after partial hepatectomy in mice

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Received: 2005-03-28 Accepted: 2005-05-03

Abstract

AIM: To investigate the *in vivo* effects of NK2 on liver regeneration after partial hepatectomy (PH).

METHODS: Survival after PH was observed with 21 NK2 transgenic mice and 23 wild-type (WT) mice over 10 d. Liver regeneration was analyzed using histology and immunohistochemistry. Expressions of genes were analyzed using Northern blot analysis, immunoprecipitation and immunoblotting, and reverse transcriptase polymerase chain reaction assay. Kaplan-Meier method and the log-rank test were used for analyzing the survival after PH. Differences in the results of immunohistochemistry and percentage of liver regeneration was determined by the Student's *t*-test.

RESULTS: More than half of NK2 transgenic mice died within 48 h after PH. After PH, increased deposition of small lipid droplets in hepatocytes was evident and hepatic proliferation was inhibited in NK2 transgenic mice. The hepatic expression and kinase activity of HGF receptor, c-Met, were unchanged among WT mice and NK2 transgenic mice after PH. The expression of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in liver tissues were prolonged in NK2 transgenic mice that died after PH.

CONCLUSION: Our findings indicate that overexpression of NK2 inhibits liver regeneration after PH.

Key words: Hepatocyte growth factor; NK2; Transgenic mice; Partial hepatectomy; Liver regeneration

Otsuka T, Horiguchi N, Kanda D, Kosone T, Yamazaki Y, Yuasa K, Sohara N, Kakizaki S, Sato K, Takagi H, Merlino G, Mori M. Overexpression of NK2 inhibits liver regeneration after partial hepatectomy in mice. *World J Gastroenterol* 2005; 11(47): 7444-7449
http://www.wjgnet.com/1007-9327/11/7444.asp

INTRODUCTION

Hepatocyte growth factor (HGF) is a multifunctional cytokine involved in proliferation, motility, and morphogenesis[1]. The effects of HGF are mediated through the tyrosine kinase receptor, c-Met[2]. HGF is reportedly the most potent mitogen for hepatocytes and acts as a trigger for liver regeneration[3,4]. Indeed, HGF knockout mice died *in utero* and the embryonic liver was reduced in size and had extensive loss of parenchymal cells[5]. In c-Met conditional knockout mice produced by using the Mx-cre transgene to introduce the mutation in the adult, liver regeneration after PH was impaired[6,7]. In c-Met conditional knockout mice produced by Cre/loxP-mediated gene targeting, recovery from necrosis induced by carbon tetrachloride (CCl_4) was impaired[8]. These data indicate that the HGF/c-Met signaling pathway plays an important role in liver regeneration and repair.

HGF is a heterodimeric glycoprotein consisting of an α chain of 60 ku and a β chain of 30 ku[9]. The α chain has an N domain and four kringle domains. HGF mRNA can undergo alternative splicing to create a truncated isoform, NK2, which consists of an N domain and the first two kringle domains of HGF[10]. NK2 is also able to bind to c-Met with relatively high affinity and is thought to be an HGF antagonist of a variety of biological activities *in vitro*[1,11-13]. *In vivo*, it has been reported that NK2 acts as an antagonist of HGF in cellular proliferation and protective effect on CCl_4 induced hepatotoxicity[14,15].

Here we have reported that NK2 inhibits hepatocyte proliferation and impairs liver regeneration after PH in our transgenic model.

MATERIALS AND METHODS

Animals

NK2 transgenic mice were generated on an albino FVB

genetic background as previously described[14]. Expressions of human NK2 cDNA was under the control of the mouse metallothionein-1 (MT-1) promoter and associated locus control regions as previously described[16]. Wild-type (WT) mice were littermates of NK2 transgenic mice. All studies were performed using 6-wk-old female mice. PH, consisting of removal of the median and left lateral hepatic lobes, was performed as previously described[17]. Survival was observed with 21 NK2 transgenic mice and 23 WT mice over 10 d. Percentage of liver regeneration was defined as follows: [wet weight of regenerating liver/body weight (g/g mouse)]/[wet weight of original liver/body weight (g/g mouse)]×100%[18]. The animal experiments were conducted in compliance with the guidelines for animal care and use established by Gunma University Graduate School of Medicine.

Histology

Mice were injected with 50 μg/g of 5-bromo-2'-deoxyuridine (BrdU, Becton Dickinson, San Jose, CA, USA) 1 h before being killed. Livers were removed before and 48 h after PH. The tissues were fixed in 10% formalin, embedded in paraffin and stained with hematoxylin-eosin (H-E) and Oil-red-O. For immunohistochemistry, slides were stained with monoclonal antibody to BrdU. At 48 h after PH, the labeled hepatocyte nuclei were scored by counting 30 high-power light microscope fields (×1 000) for each animal.

Northern blot analysis

Total RNAs from liver tissues were prepared using TRIzol (Gibco BRL, Gaithersburg, MD, USA) according to the instructions provided by the manufacturer, and 20 μg of total RNA was loaded per lane onto a 1% agarose-formaldehyde gel and transferred to a nylon membrane (Amersham Pharmacia Biotech, Piscataway, NJ, USA). As described previously, transcripts of the NK2 transgene and endogenous c-Met were detected with a 2.2-kbp mouse HGF cDNA probe and a 1.5-kbp mouse c-Met cDNA probe, respectively[15]. The membrane was rehybridized with a mouse β-actin probe (generously provided by Kojima I) to control for RNA loading and transfer variation.

Immunoprecipitation (IP) and immunoblotting (IB)

Quantification of c-Met and c-Met tyrosine phosphorylation was performed as described previously[19]. Briefly, frozen liver tissues were solubilized in RIPA buffer consisting of 50 mmol/L Tris (pH 7.4), 50 mmol/L NaCl, 1% Triton X-100, 5 mmol/L ethylenediaminetetraacetic acid, 10 mmol/L sodium PPi (Sigma, St. Louis, MO, USA), 50 mmol/L sodium fluoride (Sigma), 1 mmol/L sodium orthovanadate (Sigma), 1 mmol/L phenylmethylsulfonyl fluoride (Boehringer Mannheim, Mannheim, Germany), 10 μg/mL leupeptin (Boehringer Mannheim), 10 μg/mL pepstatin (Boehringer Mannheim, Tokyo, Japan) and 10 μg/mL aprotinin (Boehringer Mannheim). Protein concentration in the resulting lysates was determined using Albumin Standard (Pierce, Rockford, IL, USA) and equivalent amounts of lysate (200 μg) were incubated with anti c-Met antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2 h on ice. After the addition of GammaBind G Sepharose (Boehringer Mannheim) and washing in RIPA buffer, samples were fractionated on 10% polyacrylamide gels (Biocraft). After electrophoretic transfer to nitrocellulose membranes (Bio-Rad, Richmond, CA, USA), filters were blocked and then incubated with anti c-Met antibody overnight. c-Met was visualized by incubation with anti-goat antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology, CA, USA) by using enhanced chemiluminescence (Santa Cruz Biotechnology, CA, USA) according to the instructions supplied by the manufacturer. Subsequently, filters were stripped with buffer consisting of 100 mmol/L 2-mercaptoethanol (Sigma), 2% sodium dodecyl sulfate, and 62.5 mmol/L Tris-HCl (pH 6.7) at 50 ℃ for 30 min. Filters were reblocked and incubated overnight with anti-phosphotyrosine (PY) antibody (Transduction Laboratories, Lexington, KY, USA) overnight. PY was visualized using an anti-rabbit antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology, CA, USA) and enhanced chemiluminescence (Santa Cruz Biotechnology, CA, USA) according to the instructions obtained from the manufacturer.

Reverse transcriptase polymerase chain reaction (RT-PCR) assay

RT-PCR was used to measure the expression of liver TNF-α mRNA and liver IL-6 mRNA. Total RNAs from liver tissues were prepared using TRIzol as previously described[15]. cDNA was prepared from 1 μg of total RNA from each liver sample by the SuperScript Preamplification System for First Strand cDNA Synthesis kit (GIBCO BRL) according to the manufacturer's instructions. The PCR reaction contained, in the same buffer as the reverse transcriptase reaction, cDNA corresponding to 50 ng of input RNA and 2.5 U AmpliTaq DNA polymerase. It was performed at 94 ℃ for 1 min, 55 ℃ for 1 min, 72 ℃ for 1.5 min for 35 cycles. Amplified products were electrophoresed in 2% agarose gels and stained with ethidium bromide. The primer pairs used were as follows[20]. TNF-α sense, 5'-AGCCCACGTCGTAGCAAACCACCAA-3'; antisense, 5'-ACACCCATTCCCTTCACAGAGCAAT-3' (448-bp product size), IL-6 sense, 5'-TATGAAGTTCCTCTCTGCAA-3'; antisense, 5'-CTTTGTATCTCTGGAAGTTT-3' (285-bp product size), β-actin sense, 5'-GCACCACACCTTCTACAATGAG-3'; antisense, 5'-AAATAGCACAGCCTGGATAGCAAC-3' (150-bp product size).

Statistical analysis

All experimental data are shown as mean±SD. Cumulative survival was plotted by the Kaplan-Meier method, and the significance of differences was examined by the log-rank test. Differences in the index of BrdU-labeled hepatocytes and percentage of liver regeneration were determined by

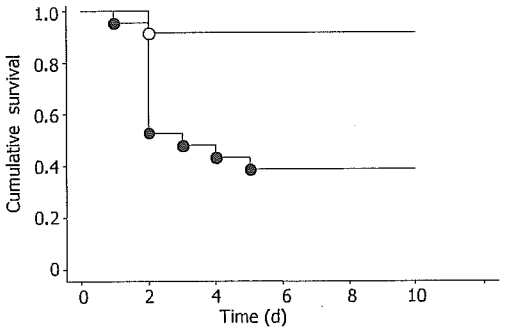

Figure 1 Cumulative survival after PH.Survival of WT mice (n = 23, open circles) and NK2 transgenic mice (n = 21, closed circles) after PH. The survival rate was significantly low in NK2 transgenic mice (^aP<0.01 *vs* WT mice).

the Student's t-test for each group. The level of significance for all statistical analyses was set at P<0.01.

RESULTS

Survival after PH

We examined the effect of NK2 on mortality of mice after PH (Figure 1). Ten of 21 NK2 transgenic mice (47.6%) died within 2 d. In contrast, 2 of 23 WT mice (8.7%) died within 2 d. Survival of NK2 transgenic mice after PH was significantly decreased compared with that of WT mice (P<0.01). Sham operation was performed using four mice per group. All four NK2 transgenic mice and all four WT mice survived until experiments were terminated after 10 d (data not shown).

Morphological alteration in liver and hepatocyte proliferation after PH

The liver structure of regenerating livers of WT mice after PH was normal (Figure 2A). NK2 transgenic mice that died during the first 48 h after PH had smooth and yellow livers. Histological examination revealed that livers of the dying NK2 transgenic mice contained a very large amount of intracellular small droplets at 48 h after PH (Figure 2B). These vesicles were readily identifiable as lipid by Oil-red-O stain (Figure 2C). BrdU staining was performed to analyze hepatocyte proliferation of each mouse at 48 h after PH (Figures 2D and E). The labeling index of the dying NK2 transgenic hepatocytes was significantly decreased relative to that of WT at 48 h after PH (WT, 18.0±6.6; dying NK2, 0.8±1.0, P<0.01) (Figure 2F). On the other hand, the percentage of liver regeneration of healthy NK2 transgenic mice and that of WT mice at 48 h after PH were 69.0±8.6% (n = 5) and 66.1±5.0% (n = 4), respectively (P = 0.59).

Expression of the transgene, endogenous c-Met and activation of c-Met in the liver after PH

High expression of transgene was detected at each time point in NK2 transgenic livers (Figure 3A). There was no difference of endogenous c-Met expression among WT mice, dying NK2 transgenic mice and healthy NK2 transgenic mice (Figure 3A). We next investigated the expression and phosphorylation of c-Met protein. The levels of c-Met protein were in accordance with the c-Met transcription findings and there was no difference of tyrosine phosphorylation of c-Met among the WT mice, dying NK2 transgenic mice and healthy NK2 transgenic mice (Figure 3B).

Hepatic expression of TNF-α and IL-6 after PH

We next examined the expressions of TNF-α and IL-6 mRNAs before PH and 48 h after PH in the livers of WT mice, dying NK2 transgenic mice and healthy NK2 transgenic mice by RT-PCR (Figure 4). TNF-α mRNA was increased in the livers of the dying NK2 at 48 h after PH. In contrast, the level of TNF-α mRNA expression at that point was similar to that before PH in WT mice and healthy NK2 transgenic mice. IL-6 mRNA was detected in the livers of the dying NK2 at 48 h after PH but not detected in WT mice and healthy NK2 transgenic mice at that point.

DISCUSSION

HGF is a potent mitogen for hepatocytes and appears to act on hepatocytes during liver regeneration after PH[3]. Indeed, HGF stimulates liver regeneration after PH in HGF transgenic mice[18,21]. NK2 is a naturally occurring HGF alternative splice variant and acts as an antagonist of HGF *in vitro*[1]. However, very little is known about the *in vivo* role of NK2 in the modulation of HGF activity. When NK2 transgenic mice, created using a mouse MT-1 promoter and associated locus control regions, were treated with zinc sulfate in water, a small reduction in liver size was observed[14]. This finding indicated that NK2 was able to inhibit endogenous HGF-mediated hepatocyte proliferation *in vivo*. Recently, we have demonstrated that overexpression of NK2 does not inhibit hepatocyte proliferation after liver damage induced by CCl_4 administration using NK2 transgenic mice[15]. In this study, we have investigated the effects of NK2 on liver regeneration after PH using NK2 transgenic mice. After PH, about half of the NK2 transgenic mice died within 2 d, whereas almost all WT mice survived that period. NK2 transgenic mice did not exhibit overt abnormal phenotypes, including the liver[15]. However, the NK2 transgenic mice that died after PH showed massive intracellular accumulation of lipid in hepatocytes at 48 h after PH. Moreover, the BrdU leveling index of hepatocytes in NK2 transgenic mice at 48 h after PH demonstrated their poor capacity to enter the S phase. Because DNA replication after PH starts at 20 to 34 h and reaches a peak at 36 to 44 h in mice[22,23], these results indicate that overexpression of NK2 inhibited DNA replication of hepatocytes after PH. The plasma HGF level was reported to peak at 48 h after PH[24]. Hepatic expression of the NK2 transgene transcript was very high at each time point after PH. This suggested that overexpression of NK2 inhibited the proliferative effect of endogeneous HGF after PH. On the other hand, the

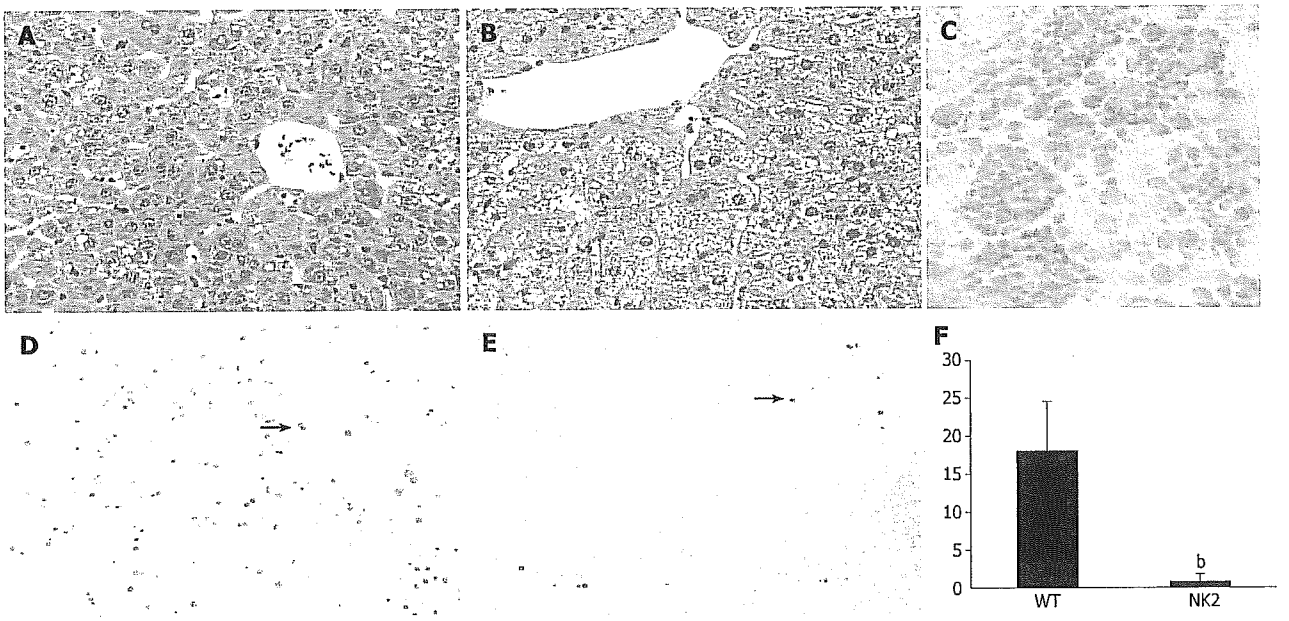

Figure 2 Liver regeneration and proliferation of hepatocytes after PH. H-E stain of WT mouse liver (**A**) and NK2 transgenic mouse liver (**B**) at 48 h after PH. NK2 transgenic mouse liver contained a large amount of intracellular small droplets. Magnification, ×200. Oil-red-O stain of NK2 transgenic mouse liver (**C**) at 48 h after PH. Note the presence of many small lipid droplets in hepatocytes. Magnification, ×400. BrdU stain of WT mouse liver (**D**) and NK2 transgenic mouse liver (**E**) at 48 h after PH. The arrows show hepatocytes undergoing DNA synthesis. Magnification, ×100. The BrdU labeling index of WT mice and NK2 transgenic mice at 48 h after PH (n = 30 per group). (**F**) Data are mean±SD of the mean. Hepatocyte proliferation was significantly reduced in NK2 transgenic mouse liver ($^bP<0.01$ *vs* WT mouse liver).

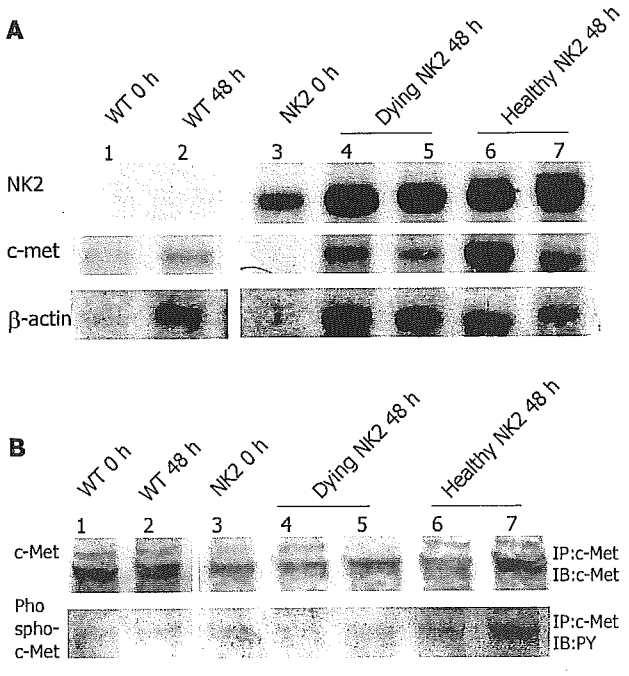

Figure 3 Expressions of RNAs and proteins of transgene and c-Met after PH. RNA expression of transgenic NK2 and c-Met, and c-Met protein levels and activity in livers from WT and NK2 transgenic mice after PH. Lane 1, WT mouse before PH; lane 2, WT mouse at 48 h after PH; lane 3, NK2 transgenic mouse before PH; lanes 4 and 5, dying NK2 transgenic mice at 48 h after PH; lanes 6 and 7, healthy NK2 transgenic mice at 48 h after PH. (**A**) Each RNA sample was subjected to Northern blot analysis. Expression levels of all transgenes were high at each time point and endogeneous c-Met expression was unaltered in each group. β-actin RNA expression was also examined as a control. (**B**) Each protein sample was subjected to IP and then IB with an anti c-Met antibody or an anti PY antibody. c-Met expression and the kinase activity were unaltered in each group.

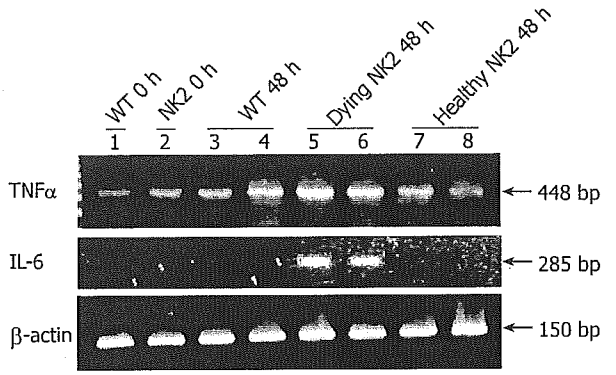

Figure 4 RNA expression of TNF-α and IL-6 after PH. TNF-α mRNA and IL-6 mRNA expression in livers of WT mice and NK2 transgenic mice. Lane 1, WT mouse before PH; lane 2, NK2 transgenic mouse before PH; lanes 3 and 4, WT mice at 48 h after PH; lanes 5 and 6, dying NK2 transgenic mice at 48 h after PH; lanes 7 and 8, healthy NK2 transgenic mice at 48 h after PH. The expression of TNF-α mRNA was increased in NK2 transgenic mice that died at 48 h after PH. IL-6 mRNA expression was detected in NK2 transgenic mice that died at 48 h after PH. β-actin mRNA expression was also examined as a control.

expression of endogenous c-Met RNA and c-Met protein, and phosphorylation of c-Met in the livers of WT and NK2 transgenic mice was unchanged at each time point after PH. Although we did not understand why only half of NK2 transgenic mice died, there could be differences in circulating levels of NK2 or critical local changes in the transgene expression[25].

Hepatocyte proliferation after PH occurs first in periportal cells, and then in perivenous cells[26]. This indicates that HGF is produced from endothelial and

Kupffer cells, and stimulates hepatocyte proliferation via a paracrine mechanism. In addition, HGF acts on hepatocytes located around the central vein by an endocrine mechanism. On the other hand, after CCl_4 administration, hepatocyte proliferation occurs randomly in the lobulus[27]. This indicates that HGF is produced by a paracrine mechanism. HGF may therefore have a differential role in hepatocyte proliferation in the PH and CCl_4 administration models. That is, NK2 overexpression might be inhibitory to hepatocyte proliferation after PH but not after CCl_4-induced acute liver injury.

TNF-α and IL-6 are known to be initiators of liver regeneration after PH[22]. Studies in TNF receptor 1 and IL-6 knock out mice demonstrated the sequence in which TNF-α is induced first after PH, followed by induction of IL-6[22]. Hepatic IL-6 mRNA expression peaked at 4 h after PH in WT mice[28]. TNF receptor 1 knock out mice showed massive lipid accumulation in hepatocytes at 40 h after PH[29] and IL-6 knock out mice showed poor hepatocyte DNA synthesis at 36 h after PH[30]. The observations in these mice are similar to those in NK2 transgenic mice after PH. We have investigated hepatic expression of TNF-α and IL-6 mRNA in NK2 transgenic mice after PH. TNF-α mRNA was increased and IL-6 mRNA was detected in the dying NK2 at 48 h after PH. This result indicates that liver regeneration was impaired in NK2 transgenic mice.

In conclusion, our study demonstrates that overexpression of NK2 prevents liver regeneration after PH.

REFERENCES

1 **Montesano R,** Soriano JV, Malinda KM, Ponce ML, Bafico A, Kleinman HK, Bottaro DP, Aaronson SA. Differential effects of hepatocyte growth factor isoforms on epithelial and endothelial tubulogenesis. *Cell Growth Differ* 1998; **9:** 355-365

2 **Bottaro DP,** Rubin JS, Faletto DL, Chan AM, Kmiecik TE, Vande Woude GF, Aaronson SA. Identification of the hepatocyte growth factor receptor as the c-met proto-oncogene product. *Science* 1991; **251:** 802-804

3 **Fausto N,** Laird AD, Webber EM. Liver regeneration. 2. Role of growth factors and cytokines in hepatic regeneration. *FASEB J* 1995; **9:** 1527-1536

4 **Phaneuf D,** Moscioni AD, LeClair C, Raper SE, Wilson JM. Generation of a mouse expressing a conditional knockout of the hepatocyte growth factor gene: demonstration of impaired liver regeneration. *DNA Cell Biol* 2004; **23:** 592-603

5 **Schmidt C,** Bladt F, Goedecke S, Brinkmann V, Zschiesche W, Sharpe M, Gherardi E, Birchmeier C. Scatter factor/hepatocyte growth factor is essential for liver development. *Nature* 1995; **373:** 699-702

6 **Borowiak M,** Garratt AN, Wustefeld T, Strehle M, Trautwein C, Birchmeier C. Met provides essential signals for liver regeneration. *Proc Natl Acad Sci USA* 2004; **101:** 10608-10613

7 **Hodgson H.** c-met-there when it's needed? *J Hepatol* 2005; **43:** 544-546

8 **Huh CG,** Factor VM, Sanchez A, Uchida K, Conner EA, Thorgeirsson SS. Hepatocyte growth factor/c-met signaling pathway is required for efficient liver regeneration and repair. *Proc Natl Acad Sci USA* 2004; **101:** 4477-4482

9 **Nakamura T,** Nishizawa T, Hagiya M, Seki T, Shimonishi M, Sugimura A, Tashiro K, Shimizu S. Molecular cloning and expression of human hepatocyte growth factor. *Nature* 1989; **342:** 440-443

10 **Chan AM,** Rubin JS, Bottaro DP, Hirschfield DW, Chedid M, Aaronson SA. Identification of a competitive HGF antagonist encoded by an alternative transcript. *Science* 1991; **254:** 1382-1385

11 **Recio JA,** Merlino G. Hepatocyte growth factor/scatter factor induces feedback up-regulation of CD44v6 in melanoma cells through Egr-1. *Cancer Res* 2003; **63:** 1576-1582

12 **Liang H,** O'Reilly S, Liu Y, Abounader R, Laterra J, Maher VM, McCormick JJ. Sp1 regulates expression of MET, and ribozyme-induced down-regulation of MET in fibrosarcoma-derived human cells reduces or eliminates their tumorigenicity. *Int J Oncol* 2004; **24:** 1057-1067

13 **Guerin C,** Luddy C, Abounader R, Lal B, Laterra J. Glioma inhibition by HGF/NK2, an antagonist of scatter factor/hepatocyte growth factor. *Biochem Biophys Res Commun* 2000; **273:** 287-293

14 **Otsuka T,** Jakubczak J, Vieira W, Bottaro DP, Breckenridge D, Larochelle WJ, Merlino G. Disassociation of met-mediated biological responses in vivo: the natural hepatocyte growth factor/scatter factor splice variant NK2 antagonizes growth but facilitates metastasis. *Mol Cell Biol* 2000; **20:** 2055-2065

15 **Otsuka T,** Takagi H, Horiguchi N, Toyoda M, Sato K, Takayama H, Mori M. CCl4-induced acute liver injury in mice is inhibited by hepatocyte growth factor overexpression but stimulated by NK2 overexpression. *FEBS Lett* 2002; **532:** 391-395

16 **Palmiter RD,** Sandgren EP, Koeller DM, Brinster RL. Distal regulatory elements from the mouse metallothionein locus stimulate gene expression in transgenic mice. *Mol Cell Biol* 1993; **13:** 5266-5275

17 **Webber EM,** Wu JC, Wang L, Merlino G, Fausto N. Overexpression of transforming growth factor-alpha causes liver enlargement and increased hepatocyte proliferation in transgenic mice. *Am J Pathol* 1994; **145:** 398-408

18 **Sakata H,** Takayama H, Sharp R, Rubin JS, Merlino G, LaRochelle WJ. Hepatocyte growth factor/scatter factor overexpression induces growth, abnormal development, and tumor formation in transgenic mouse livers. *Cell Growth Differ* 1996; **7:** 1513-1523

19 **Horiguchi N,** Takayama H, Toyoda M, Otsuka T, Fukusato T, Merlino G, Takagi H, Mori M. Hepatocyte growth factor promotes hepatocarcinogenesis through c-Met autocrine activation and enhanced angiogenesis in transgenic mice treated with diethylnitrosamine. *Oncogene* 2002; **21:** 1791-1799

20 **Tozawa K,** Hanai H, Sugimoto K, Baba S, Sugimura H, Aoshi T, Uchijima M, Nagata T, Koide Y. Evidence for the critical role of interleukin-12 but not interferon- gamma in the pathogenesis of experimental colitis in mice. *J Gastroenterol Hepatol* 2003; **18:** 578-587

21 **Shiota G,** Wang TC, Nakamura T, Schmidt EV. Hepatocyte growth factor in transgenic mice: effects on hepatocyte growth, liver regeneration and gene expression. *Hepatology* 1994; **19:** 962-972

22 **Fausto N.** Liver regeneration. *J Hepatol* 2000; **32:** 19-31

23 **Tzung SP,** Fausto N, Hockenbery DM. Expression of Bcl-2 family during liver regeneration and identification of Bcl-x as a delayed early response gene. *Am J Pathol* 1997; **150:** 1985-1995

24 **Pediaditakis P,** Lopez-Talavera JC, Petersen B, Monga SP, Michalopoulos GK. The processing and utilization of hepatocyte growth factor/scatter factor following partial hepatectomy in the rat. *Hepatology* 2001; **34:** 688-693

25 **Yu Y,** Merlino G. Constitutive c-Met signaling through a nonautocrine mechanism promotes metastasis in a transgenic transplantation model. *Cancer Res* 2002; **62:** 2951-2956

26 **Sakai H,** Tsukamoto T, Yamamoto M, Shirai N, Iidaka T, Yanai T, Masegi T, Tatematsu M. Differential effects of partial hepatectomy and carbon tetrachloride administration on induction of liver cell foci in a model for detection of initiation activity. *Jpn J Cancer Res* 2001; **92:** 1018-1025

27 **Ishiki Y,** Ohnishi H, Muto Y, Matsumoto K, Nakamura T. Di-

rect evidence that hepatocyte growth factor is a hepatotrophic factor for liver regeneration and has a potent antihepatitis effect in vivo. *Hepatology* 1992; **16:** 1227-1235

28 **Yamada Y,** Kirillova I, Peschon JJ, Fausto N. Initiation of liver growth by tumor necrosis factor: deficient liver regeneration in mice lacking type I tumor necrosis factor receptor. *Proc Natl Acad Sci USA* 1997; **94:** 1441-1446

29 **Yamada Y,** Webber EM, Kirillova I, Peschon JJ, Fausto N. Analysis of liver regeneration in mice lacking type 1 or type 2 tumor necrosis factor receptor: requirement for type 1 but not type 2 receptor. *Hepatology* 1998; **28:** 959-970

30 **Aldeguer X,** Debonera F, Shaked A, Krasinkas AM, Gelman AE, Que X, Zamir GA, Hiroyasu S, Kovalovich KK, Taub R, Olthoff KM. Interleukin-6 from intrahepatic cells of bone marrow origin is required for normal murine liver regeneration. *Hepatology* 2002; **35:** 40-48

Science Editor Guo SY **Language Editor** Elsevier HK

Abdominal Imaging

Published online: 1 February 2005

Abdom Imaging (2005) 30:306–313
DOI: 10.1007/s00261-004-0270-8

Short-term complications of retrograde transvenous obliteration of gastric varices in patients with portal hypertension: effects of obliteration of major portosystemic shunts

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Abstract

The type, incidence, and severity of complications of balloon-occluded retrograde transvenous obliteration (B-RTO) for gastric varices should be precisely estimated. Complications were evaluated in 38 patients who had fundic gastric varices and 43 B-RTO procedures during injection of ethanolamine oleate (phase 1), within 4 h after injection (phase 2), 24 h after injection (phase 3), and from 24 h to 10 days after injection (phase 4). Endoscopic evaluation at 8 weeks showed resolution of gastric varices in 35 of 38 patients (92%) and smaller varices in the remaining three (8%). B-RTO caused transient hypertension in 35% of patients, hemoglobinuria in 49%, and fever in 33% during phases 1, 2, and 3, respectively. Pleural effusion, pulmonary infarction, ascites, gastric ulcers with unique appearance, localized mosaic-like change of gastric mucosa, and hemorrhagic portal hypertensive gastropathy were noted in phase 4. There were no fatalities. Lactate dehydrogenase, aspartate aminotransferase, and bilirubin increased on day 1. Each datum was retrieved within 7 days. The severity of lactate dehydrogenase elevation correlated significantly with the volume of infused ethanolamine oleate. Thus, B-RTO is a safe and effective management of fundic varices. However, short-term hemodynamic change after B-RTO may cause gastric mucosal damage. Pulmonary infarction and pleural effusion are potential complications.

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Key words: Balloon-occluded retrograde transvenous obliteration—Gastric varices—Short term—Complication—Portal hypertension

Bleeding from gastric varices is a serious complication of portal hypertension and a serious medical emergency. Gastric varices bleed more profusely and are more likely to rebleed than esophageal varices [1]. The natural history of patients who have cirrhosis and fundic varices is adversely influenced by hemorrhage from fundic varices. Thus, in addition to urgent and elective treatment, prophylactic obliteration of high-risk large fundic varices has been recommended [2]. However, in most guidelines, there is no clear indication on whether and how to treat this problem. The optimal approach for fundic varices was not codified before the introduction of balloon-occluded retrograde transvenous obliteration (B-RTO) as a management procedure [3]. There are many optional procedures, such as endoscopic variceal ligation [4], transjugular intrahepatic portosystemic shunt (TIPS) [5], percutaneous transhepatic obliteration [6], transileocecal obliteration [6], and endoscopic sclerotherapy with [7] or without [8] cyanoacrylate. Nevertheless, B-RTO has been extensively applied in the past decade for the management of fundic gastric varices in several specialized centers. The technique provides good control of gastric varices with gastrorenal or gastrocaval collaterals and has been considered to be a feasible alternative to TIPS, although the nomenclature has varied among the reporters [3, 9–11]. Further, B-RTO may be applicable for gastric varices even in patients who have poor hepatic function and hepatic encephalopathy [11–13]. B-RTO

increases hepatic portal blood flow, which may increase liver function [14, 15]. Development of esophageal varices after occlusion of the gastrorenal shunt has been reported as a long-term complication of B-RTO [16]. In addition, hemoglobinuria and fever are short-term complications [12]. One case of cardiogenic shock, caused by a mixture of ethanolamine oleate and iopamidol, has been reported [11]. Chikamori et al. [13] reported one case treated with this procedure that later developed gastritis with hemorrhage. Thus, no other warning or conspicuous systemic complications have been reported thus far [3, 9–13, 16].

The procedure of B-RTO consists of obliteration of the major portosystemic shunt in patients who have portal hypertension. This may lead to a marked change in the hemodynamics in the gastric mucosa, esophagus, spleen, intestine, liver, and systemic circulation. B-RTO can worsen portal hypertension by obliterating portosystemic shunts [15]. B-RTO creates a large thrombus not only in gastric varices but also in their in-flow and out-flow shunts. This thrombus may form very close to the major venous system such as the left renal vein or inferior caval vain and close to the major portal system including the splenic vein and portal trunk. These events may result in major complications, although actual clinical complications are indistinct in this aspect. The procedure of B-RTO is still under evaluation and has not been clearly defined in its details of technical procedure, and potential risks could be various and severe. Available reports have not stated important side effects, excluding those cited above, and prospective analyses of large series are unavailable. This study aims to fill this gap.

In the present study, we evaluated the incidence and severity of all complications encountered in the course of the procedure and during hospital stay after B-RTO.

Materials and methods

Patients

Forty-three B-RTO procedures were performed in 38 patients (21 male, 17 female) who had portal hypertension and gastric fundic varices between December 1995 and July 2000 in Maebashi Red Cross Hospital (Maebashi, Japan). Patients who had Child class C cirrhosis with ascites were excluded from this study because B-RTO may increase portal pressure [15]. Written informed consent was obtained from all patients. The main clinical characteristics of these patients are listed in Table 1. Liver cirrhosis was diagnosed in 37 patients. One patient without cirrhosis had left-side portal hypertension, which was caused by splenic vein obstruction after traumatic pancreatitis. Twenty-seven of 38 patients (71%) had associated esophageal varices and 13 patients had hepatocellular carcinoma. Computed tomography (CT) or ultrasonography excluded the presence of portal tumor thrombus in all patients who had hepatocellular carcinoma. The form of fundic varices was classified into three categories (Table 1): F1, tortuous varices; F2, moderately enlarged nodular varices; and F3, massive tumor-like varices. Nineteen patients had a history of variceal bleeding. Endoscopic variceal ligation was achieved in five of seven patients who had active bleeding at the time of urgent endoscopy. Clipping was performed in two patients. Hemostasis was secured in all seven patients. Injection sclerotherapy was not performed. Six patients underwent subsequent B-RTO within 24 h as emergent cases. This study also included 13 patients with elective B-RTO and 19 with prophylactic B-RTO.

Table 1. Characteristics of patients (n = 38)

Male/female	21/17
Age (years), mean ± standard deviation (range)	61.1 ± 11.2 (34-80)
Etiology of portal hypertension	
LC (Alcohol)	7
LC (HBV)	2
LC (HCV)	22
LC (HBV and HCV)	1
LC (non-B and non-C)	4
Primary biliary cirrhosis	1
Splenic vein obstruction[a]	1
Child-Pugh score	
Mean ± standard deviation	6.37 ± 1.17
A (5–6)	23
B (7–9)	15
C(10–15)	0
Esophageal varices: 0/F1/F2/F3	11/15/12/0
Gastric varices: F1/F2/F3	3/20/15

[a]Post-traumatic pancreatitis
HBV, HbsAg positive; HCV, anti-hepatitis C virus Ab positive; LC, liver cirrhosis

B-RTO procedure

A 6.5-French balloon catheter (Create Medic, Yokohama, Japan) was inserted in the gastrorenal shunt, gastrocaval shunt, or both through the femoral or internal jugular vein under local anesthesia. Balloon-occluded retrograde transvenous varicerography was obtained in advance. Four thousand units of haptoglobin (Welfide, Osaka, Japan) was administered by drop infusion just before treatment. After identification of gastric varices and their associated in-flow and draining vessels, the balloon was inflated with CO_2. Eighteen patients had two or more varices-associated in-flow shunts. Total numbers of in-flow shunts were 29 posterior gastric veins, 23 left gastric veins, and eight short gastric veins. Five percent solution of ethanolamine oleate with iopamidol (5% EOI) that contained equal amounts of 10% ethanolamine oleate (Grelan, Tokyo) and iopamidol 300 (Schering, Berlin, Germany) was slowly infused through the catheter into the gastric varices and their in-flow shunts in a retrograde manner during balloon occlusion. After 30 min, hemolyzed blood and excess EOI were collected through the catheter and then the balloon was deflated. In 12 patients, microcoils were used in addition to 5% EOI to embolize out-flow minor collaterals.

Evaluation of efficacy of B-RTO

We observed the site endoscopically at 1, 4, and 8 weeks after the procedure and evaluated the efficacy of B-RTO at 8 weeks in all 38 patients. Five patients (13%) who showed no improvement of varices by endoscopic investigation at 4 weeks after treatment underwent additional B-RTO procedures.

Subjective symptoms and objective findings

Complications, if any, were examined during the following intervals: phase 1, initial 30 min during balloon occlusion and injection of sclerosant; phase 2, within 4 h after balloon deflation; phase 3, within 24 h after B-RTO; and phase 4, from 24 h through 10 days. In phase 1, while blood flow of the varices was blocked by the balloon and 5% EOI was permeated through, arterial blood pressure was measured every 2 min and patients were asked to report any symptoms every 5 to 10 min. Blood pressure and body temperature was measured every 8 h for the following 72 h. Chest roentgenogram was performed before and on the day after B-RTO. Technical complications during the procedure were carefully noted.

Changes in laboratory data

Hepatic and renal function tests including total bilirubin (T-Bil), indirect bilirubin (I-Bil), alanine aminotransferase, aspartate aminotransferase (AST), lactate dehydrogenase (LDH), choline esterase (ChE), blood ammonia, serum creatinine, serum urea nitrogen, platelet count, and percentage of prothrombin activity were analyzed 1 day and 1 week after B-RTO. Patients who required blood transfusion within 48 h before B-RTO or urgent B-RTO were excluded from the laboratory data evaluation study. Twenty-six patients were entered into this study.

Statistical analysis

Results are expressed as mean ± standard deviation unless otherwise stated. The correlation between increased LDH and infused amount of 5% EOI was tested by calculating the Pearson correlation coefficient (r). Analysis of multiple comparisons was performed by repeated measure one-way analysis of variance followed by Fisher PLSD test. The level of statistical significance was set at $p < 0.05$.

Results

Efficacy of B-RTO

Endoscopic investigation at 8 weeks after B-RTO demonstrated the disappearance of gastric varices in 35 of 38 patients (92%), and smaller varices were observed in the remaining three (8%). No patient died and no patient manifested bleeding or rebleeding from gastric varices within the 2-month follow-up period.

Fig. 1. Leakage of 5% EOI from the left inferior phrenic vein and spread along the diaphragm (*arrows*). No intraperitoneal or intrathoracic hemorrhage was noted.

Complications of the B-RTO procedure

Technical complications

There were three technical complications in 43 B-RTO procedures. All occurred during 5% EOI infusion. The balloon ruptured in one patient when about 27 mL of 5% EOI had been just infused through the gastrorenal shunt. EOI was washed away immediately from the gastric varices through the left renal vein to the inferior vena cava. The patient complained of chest discomfort. Hemoglobinuria appeared within approximately 20 min and lasted for about 5 h. However, liver and renal functions remained unchanged (data not shown). B-RTO was repeated 4 weeks later. In another patient who had alcoholic liver cirrhosis, extravasation of 5% EOI was noted just after the injection, which spread gradually along the left diaphragm (Fig. 1). The patient complained of chest oppression and back pain. The varix was eradicated successfully despite this complication. In another patient, the balloon portion of the catheter slipped off the gastrorenal shunt and was displaced into the left renal vein when just after starting the 5% EOI injection. B-RTO was successfully completed on the same day by infusing

Table 2. Subjective symptoms (n = 43)

Phase 1: initial 30 min during balloon occlusion		
Chest or epigastric pain	24	(55.8%)
Nausea or vomiting	9	(20.9%)
Cold sweat	5	(11.6%)
Chest or epigastric discomfort	4	(9.3%)
Chest oppression	3	(7.0%)
Back pain	2	(4.7%)
Phase 2: within 4 h after balloon deflation		
Chest or epigastric discomfort	7	(16.3%)
Nausea or vomiting	4	(9.3%)
Chest or epigastric pain	3	(7.0%)
Phase 3: within 24 h		
Chest or epigastric discomfort	4	(9.3%)
Lumbago	4	(9.3%)
Nausea or vomiting	3	(7.0%)
Dyspnea	1[a]	(2.3%)
Phase 4: From 24 h through day 10		
Appetite loss	5	(11.6%)
Sense of abdominal distention	1[b]	(2.3%)
Diarrhea	1	(2.3%)
Tarry stool	1[c]	(2.3%)

[a]Accompanied by plumonary infarction
[b]Ascites
[c]Hemorrhagic portal hypertensive gastropathy

5% EOI more slowly. This patient had a prominent but tapering wedge-shaped gastrorenal shunt. The left renal vein was not affected and renal function did not change.

Clinical complications

Subjective symptoms reported within 10 days of B-RTO are listed in Table 2, and the objective findings are listed in Table 3. During phase 1, the major complaints were epigastralgia and chest pain. More than 20% of patients complained of nausea but vomiting was reported by only one patient. Just after the infusion of 5% EOI, 35% of patients developed transient hypertension. During phase 2, hemoglobinuria was observed in approximately 50% of the patients despite haptoglobin administration. During phase 3, the most frequent complication was fever. During phase 4, pleural effusion was noted on chest roentgenogram in five patients (Fig. 2) but disappeared within 7 days in all patients. One patient complained of mild dyspnea the next morning; arterial partial pressure of oxygen was low (53.8 mmHg), necessitating further investigation including chest CT. A small wedge-shape lesion was detected in the right lung, with pleural effusion (Fig. 3) suggestive of pulmonary infarction.

Ascites developed in three patients, which was mild in only two. Massive ascites with abdominal distention was noted in one patient, a 58-year-old male who had alcoholic cirrhosis. The patient complained of abdominal fullness and an inability to eat to satisfy his appetite for 10 days. However, 2 weeks after B-RTO, ascites began to decrease significantly by conservative treatment including a low-salt diet and administration of diuretics.

Table 3. Objective findings (n = 43)

Phase 1: initial 30 min during balloon occlusion		
Elevation or arterial blood pressure[a]	15	(34.9%)
Phase 2: within 4 h after balloon deflation		
Hemoglobinuria	21	(48.8%)
Phase 3: within 24 h		
Fever (> 38.0°C)	14	(32.6%)
Hepatic encephalopathy	1[b]	(2.3%)
Phase 4: from 24 h through day 10		
Localised mosaic-like gastric mucosa	39	(90.7%)
Pleural effusion	5	(11.6%)
Gastric ulcer	4	(9.3%)
Ascites	3	(7.0%)
Pulmonary infarction	1	(2.3%)
Hemorrhagic portal hypertensive gastropathy	1	(2.3%)

[a]Higher than 180 mmHg or elevation by more than 50 mmHg
[b]Grade II

Follow-up endoscopic examination on postoperative day 7 displayed the development of small gastric ulcers in four patients (Fig. 4). These were located at the top of nodular gastric varices. Each ulcer was surrounded by a fine reticular pattern separating areas of raised congested mucosa (mosaic-like pattern). In one patient, ulceration was accompanied by a fresh blood clot in the stomach and a small clot at the bottom of the ulcer, which indicated gastric hemorrhage from the ulcer induced by B-RTO. Blood transfusion was not needed in this patient. All ulcers showed rapid healing without apparent fold convergency formation by conservative therapy in about 1 week. The localized mucosal changes with a mosaic-like pattern and congestion in the area of treated gastric varices were observed in 39 of 43 B-RTO procedures (91%) including the four ulcers described above. All 35 patients in whom gastric varices were completely eradicated showed the mosaic-like pattern on the treated varices. One patient complained of tarry stool 6 days after B-RTO. Endoscopic examination showed exacerbation of portal hypertensive gastropathy (PHG) and diffuse oozing in the antral region. There were no ulcers or erosions. Oozing was resistant to conservative treatment. Repeated series of sclerotherapy with polidocanol were needed for hemostasis.

Changes in laboratory data

Laboratory data are presented in Table 4. LDH, AST, T-Bil, and I-Bil were increased on day 1 and returned to normal on day 7. LDH isozyme 1 + 2, which is influenced by hemolysis, was 67.4 ± 7.1% on day 1. Serum alanine aminotransferase remained unchanged on day 1. To further investigate the influence of hemolysis on LDH, we assessed the relation between LDH and the administered volume of 5% EOI, which is potent inducer of hemolysis [17]. The amount of infused 5% EOI was 27.6 ± 12.8 mL. The increase in LDH ($LDH_{day\ 1} - LDH_{day\ 0}$) correlated significantly with the infused amount of 5% EOI (Fig. 5). Levels of serum creatinine, serum urea nitrogen, platelet count, ChE, and blood

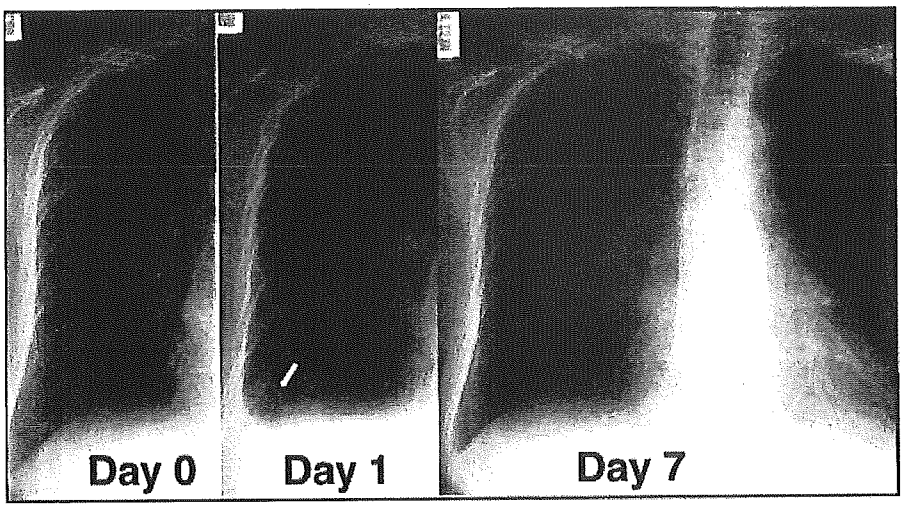

Fig. 2. Chest roentgenogram showing pleural effusion (*arrow*) on the day after B-RTO. Effusion was not associated with clinical symptoms such as cough or chest pain.

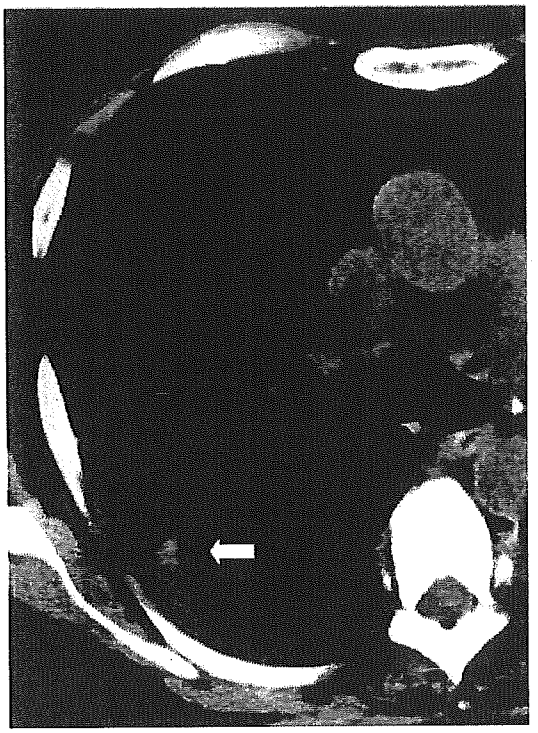

Fig. 3. Chest CT showing a small pulmonary infarct in the right lobe (*arrow*) accompanied by mild pleural effusion.

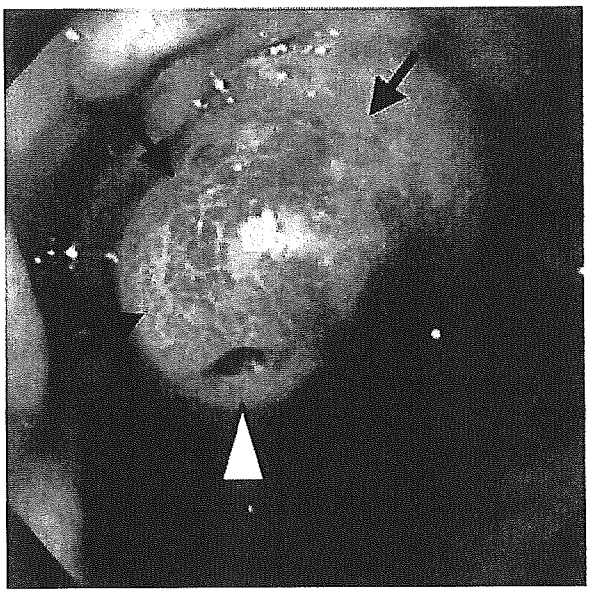

Fig. 4. Endoscopic examination demonstrating a small gastric ulcer (*arrowhead*) on the top of a successfully treated gastric varix on postoperative day 7. Fresh blood clots are present in the stomach. Note the small clot at the bottom of the ulcer. The ulcer was surrounded by mosaic-like pattern of mucosal change, i.e., localized PHG (*arrows*).

ammonia did not change in our patients who had been treated with B-RTO.

Discussion

B-RTO is an effective therapy for gastric fundic varices [3, 9–16]. We confirmed the efficacy of this procedure in 35 of 38 gastric varices (92%) and reported novel and significant complications in detail.

Pulmonary glue emboli may occur after gastric variceal sclerotherapy [18]. Gastric varices generally drain directly into a large vein such as the left renal vein or inferior vena cava and lack drainage into intervening small veins. Therefore, sclerosant or fragments of blood clots could flow out and lodge into the pulmonary artery from a gastrorenal shunt or gastrocaval shunt after balloon deflation. However, only one patient developed pulmonary infarction as demonstrated by symptoms and