Original Paper

Oxidative Stress and Tumor Progression in Colorectal Cancer

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ABSTRACT

Background/Aims: Elevated oxidative status has been found in many types of cancer cells. Recent studies have shown that the enzymatic product of thymidine phosphorylase (TP) generated reactive oxygen species (ROS) within cancer cells. The aim of this study was thus to evaluate the signal transduction pathway and the role of ROS in colorectal cancer.

Methodology: Blood specimens were obtained from the drainage vein of the tumor during operation in 76 patients with colorectal cancer. Serum ROS levels were measured using the derivative-Reactive Oxygen Metabolites (d-ROM) test and serum TP levels were examined by a highly sensitive ELISA method. Results: There was no significant correlation between serum levels of ROS and TP. Serum ROS levels were elevated in proportion to tumor invasion and had a significant positive correlation with tumor size ($p<0.05$). However, they did not increase in patients with liver metastasis.

Conclusions: These findings suggest that ROS are independent of TP-triggered signaling transduction and are associated with increased tumor invasion, but not liver metastasis in patients with colorectal cancer. From this point of view, new strategies related to ROS may provide improved therapeutic results as well as a preventative effect on carcinogenesis of the colorectum.

KEY WORDS: Reactive oxygen species (ROS); Thymidine phosphorylase (TP), colorectal cancer; Derivative-reactive oxygen metabolites (d-ROM)

ABBREVIATIONS: Reactive Oxygen Species (ROS); Thymidine Phosphorylase (TP); Derivative-Reactive Oxygen Metabolites (d-ROM)

INTRODUCTION

Reactive oxygen species (ROS) include superoxide radical anion (O_2-), hydroxyl radical (· OH), and hydrogen peroxide (H_2O_2) and play important roles in cell growth and intracellular signal transduction pathways (1-5). Excessive production of intracellular ROS has been reported to result in an oxidative environment which damages cellular molecules or modulates gene expression (6-8).

The colon is constantly exposed to ROS originating from endogenous and exogenous sources (9). It is well known that enhanced oxidative stress of the colon is associated with an increased risk of cancer (10-12). In addition, ROS have been proposed to be involved in tumor metastasis, which is a complicated processes including epithelial-mesenchymal transition (EMT), migration, invasion of the tumor cells and angiogenesis (13, 14). A more comprehensive understanding of ROS-triggered signaling transduction, transcriptional activation and regulation of gene expression will help strengthen our understanding of the critical role of ROS in tumor progression.

Recent studies have reported that the enzymatic product of thymidine phosphorylase (TP), an enzyme involved in pyrimidine metabolism, generates ROS within a TP-overexpressing cancer cell and free radical stress increases tumor cell production of angiogenic factors and an intestinal collagenase (14-16). In our previous studies, we showed that the serum TP levels in venous blood drainage specimens reflect the prognosis of patients with colorectal cancer, particularly the risk of liver metastasis (17, 18). However, it remains unclear why the serum TP levels are elevated in patients with liver metastasis. Accordingly, the purpose of the present study was to investigate whether an oxidative stress mechanism is regulated by TP and to evaluate the role of ROS in colorectal tumor progression.

METHODOLOGY

Patients and Tumor Samples

Seventy-six patients (47 males and 29 females) with colorectal cancer, who underwent surgery between June 1997 and January 2002 in the Department of Surgery, Nagasaki University, were enrolled as subjects **(Table 1)**. None had received chemotherapy or irradiation prior to surgery. Twelve patients had liver metastases (10 patients with synchronous metastasis and 2 patients with metachronous metastasis). The average age at surgery was 65.3 years (range, 34-87 years). The average follow-up period was 89.4 months. The extent of tumor invasion was based on TNM classification. Five patients were classified as T1, 7 as T2, 55 as T3, and 9 as T4. Thirty-three patients had lymph node metastases. Serum samples were collected after obtaining informed con-

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TABLE 1. Patient Characteristics

Characteristics		No. of patients	%
Age (yr)	Median	65.3	
	Range	34-87	
Gender	Male	47	62
	Female	29	38
Tumor site	Colon	41	54
	Rectum	35	46
Depth of invasion	T1	5	7
	T2	7	9
	T3	55	72
	T4	9	12
Tumor size	≤ 40 mm	33	43
	> 40 mm	43	57
Lymph nodes metastasis	(-)	43	57
	(+)	33	43
Liver metastasis	(-)	64	84
	(+)	12	16

sent from each patient in accordance with institutional guidelines. All patients had histologically verified adenocarcinoma of the colon or rectum.

Clinical Follow-Up

All patients were followed up after discharge by physical examination, routine serum chemistries and serum tumor marker tests every 1-3 months and by abdominal ultrasonography and computed tomography every 3-6 months.

Measurement of the Serum TP Levels

The serum samples were obtained from venous blood drainage specimens of the primary tumor immediately after laparotomy, and then were stored at -80°C until use. The serum TP levels were determined by enzyme-linked immunosorbent assay (ELISA) using the previously reported method with the following modifications (17, 18). The same pair of monoclonal antibodies - 104B and 232-2 - was used, but 232-2 was labeled with biotin and its binding was detected by peroxidase-conjugated streptavidin. Due to these modifications, the accuracy of the ELISA was improved 25-fold in comparison to the original procedure. In serum from the peripheral blood of 16 healthy volunteers, the mean TP level was 14.1±5.2ng/ml.

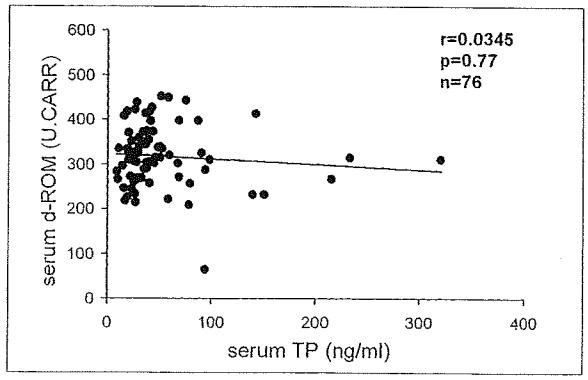

FIGURE 1 Correlation between the serum thymidine phosphorylase (TP) levels and the serum derivatives of reactive oxygen metabolites (d-ROM) levels in venous blood drainage specimens from colorectal cancer patients.

Measurement of the Serum d-ROM Levels

The oxidative status was studied by measuring hydroperoxides in the serum using the d-ROM (derivatives of reactive oxygen metabolites) test. The test was carried out using the Free Radical Analytical System 4 (FRAS4; Wismerll Co. Ltd. Tokyo, Japan). The test is based on the concept that the amount of organic hydroperoxides present in serum is related to the free radicals from which they are formed. When the serum sample is dissolved in an acidic buffer, the hydroperoxides react with the transition metal ions liberated from the proteins in the acidic medium and are converted to alkoxy and peroxy radicals. These newly formed radicals are able to oxidize an additive (N, N-diethyl-para-phenylendiamine) to the corresponding radical cation. It was reported that the value of serum ROS had a high positive correlation with the serum d-ROM value of measured by the d-ROM test (19-21). The concentration of this persistent species can be easily determined through spectrophotometric procedures (absorption at 505 nm), and are generally expressed in conventional units (Carratelli units; U.CARR), in which 1U.CARR corresponds to 0.8mg/lH_2O_2. The normal values of the test are less than 300U.CARR. Values>300U.CARR indicate a condition of oxidative stress (301-320U.CARR: borderline, 321-340U.CARR: mild oxidative stress, 341-400U.CARR: moderate oxidative stress, 401-500U.CARR: strong oxidative stress, 501U.CARR and higher: very strong oxidative stress).

Statistical Analyses

All data were expressed as the means±standard deviation. The Mann-Whitney test was used to determine statistically significant differences between two groups. Spearman's correlation was used to examine correlations. Differences were considered to be statistically significant when $p<0.05$.

RESULTS

Relationship between the serum levels of d-ROM and TP

The serum d-ROM and TP levels in venous blood drainage specimens were successfully measured in all patients in this study. The mean of the serum d-ROM levels was 317.4±68.6U.CARR, and the mean of the serum TP levels was 52.1±51.9ng/ml. As shown in **Figure 1**, the serum d-ROM levels were not significantly correlated with the serum TP levels (p=0.77).

Relationship between the serum d-ROM and TP levels and tumor growth

First, the correlation between the serum d-ROM or TP levels and the clinicopathologic features was

evaluated. A statistically significant correlation was observed between the serum d-ROM levels and tumor size (p=0.036). The serum d-ROM levels were 329.9±77.1U.CARR in patients with a tumor of more than 40mm and 301.0±52.5U.CARR in patients with a tumor of less than 40mm **(Figure 2A)**. No significant correlation was noted between the serum TP levels and tumor size **(Figure 2B)**.

The serum d-ROM levels of each depth of invasion group are shown in Figure 3A. The serum d-ROM levels were 291.4±78.8U.CARR in the T1 group, 305.1±38.1U.CARR in the T2 group, 320.1±72.8U.CARR in the T3 group, and 324.9±59.1U.CARR in the T4 group. As the tumor invasion progressed, there was a trend toward a greater increase in the serum d-ROM levels. On the other hand, there was no correlation between the serum TP levels and the depth of invasion **(Figure 3B)**. None of the other variables, including age, gender, tumor site, and lymph node metastasis, showed a statistically significant correlation with the serum d-ROM or TP levels.

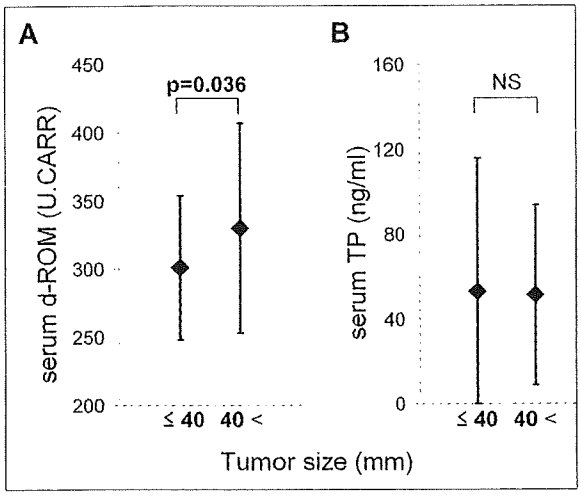

FIGURE 2 Relationship between the serum derivatives of reactive oxygen metabolites (d-ROM) levels (A) and the serum thymidine phosphorylase (TP) levels (B) and tumor size. Data points represent the means± standard deviation. NS, not significant.

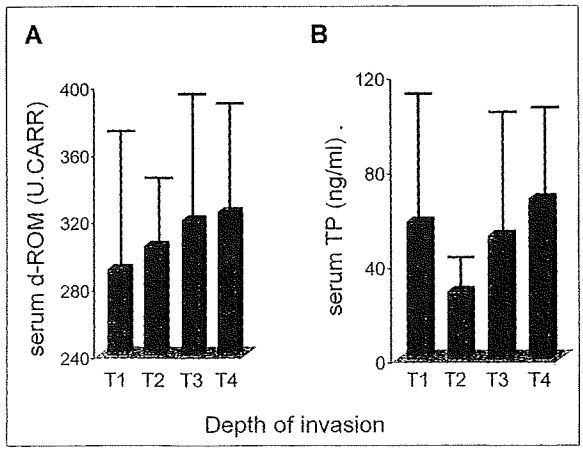

FIGURE 3 Relationship between the serum derivatives of reactive oxygen metabolites (d-ROM) levels (A) and the serum thymidine phosphorylase (TP) levels (B) and depth of invasion of colorectal cancer. Bars represent the standard deviation.

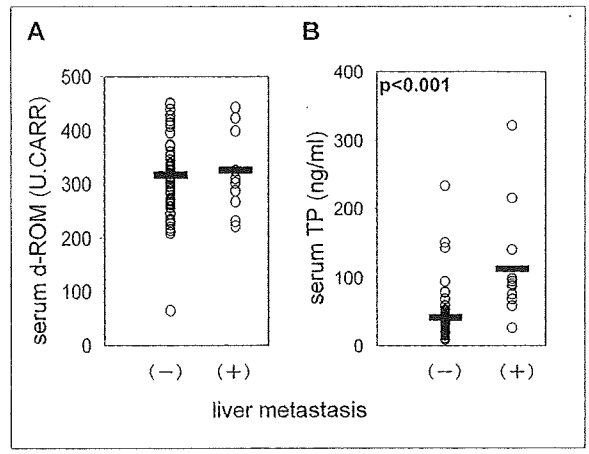

FIGURE 4 Plot depicting the serum derivatives of reactive oxygen metabolites (d-ROM) levels **(A)** and the serum thymidine phosphorylase (TP) levels **(B)** according to presence or absence of liver metastasis. Points (○) are the observed serum d-ROM or TP levels in individual patients. Bars are the mean values for each group.

Serum d-ROM and TP levels and liver metastasis

The serum d-ROM levels in the 12 patients with liver metastasis were not significantly correlated with those in the 64 patients without liver metastasis (326.1±73.5U.CARR *vs.* 315.8±68.2U.CARR) **(Figure 4A)**.

On the other hand, the serum TP levels in the patients with liver metastasis were significantly higher than those in the patients without liver metastasis (111.6±81.1ng/ml *versus* 40.9±35.5ng/ml: p<0.001) **(Figure 4B)**.

DISCUSSION

We previously indicated that serum TP levels were significantly elevated in colorectal cancer patients with liver metastasis and reflected the prognosis (17, 18). TP, which is identical to platelet-derived endothelial cell growth factor, is an angiogenic factor and catalyzes the reversible phosphorolysis of thymidine, deoxyuridine and their analogs (22-24). However, it remains uncertain how TP associates with the cascade of liver metastasis in colorectal cancer.

Recent studies have reported that 2-deoxy-D-ribose-1-phosphate (2dDR1P) released from thymidine by TP generates oxygen radicals, and that free radical oxidative stress causes angiogenesis and metastasis (14, 16). ROS can stimulate cell proliferation, promote genetic instability, and induce adaptive responses that enable cancer cells to maintain their malignant phenotypes. ROS-mediated DNA damage has long been thought to play an important role in carcinogenesis initiation and malignant transforma-

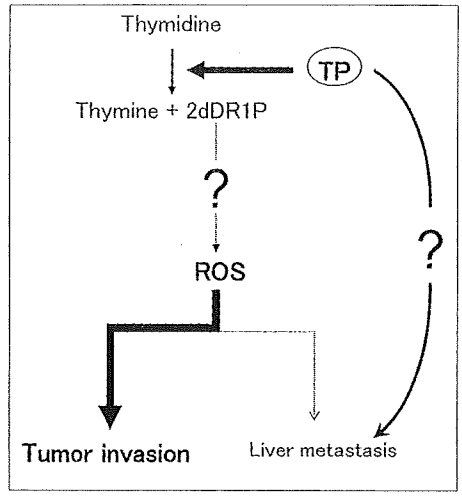

FIGURE 5
Schematic representation of the roles of thymidine phosphorylase (TP) and reactive oxygen species (ROS) in tumorigenesis.

tion by leading to mutations in tumor suppressor genes (25). The gastrointestinal tract, especially the colon, is constantly exposed to ROS originating from endogenous and exogenous sources, and ROS have been associated with an increased risk of colon cancer (14, 26).

Therefore, we hypothesized that a TP-triggered signal transduction pathway plays a role in the liver metastasis of patients with colorectal cancer. The mechanism of this association would proceed as follows. (1)The downstream mediators of TP function give rise to oxidative stress. (2)ROS increase tumor cell production of the angiogenic factors interleukin-8 (IL-8) and vascular endothelial growth factor (VEGF), in addition to inducing production of matrix metalloproteinase-1 (MMP-1). This gives rise to (3)liver metastasis of the colorectal cancer.

To examine this hypothesis, we measured serum ROS levels using the d-ROM test and compared them with serum TP levels. The results showed that there was no significant correlation between the serum levels of ROS and TP. In addition, serum ROS levels were not significantly elevated in colorectal cancer patients with liver metastasis. In contrast, serum ROS levels were elevated in proportion to tumor invasion and had a significant positive correlation with tumor size. These results suggest that TP may be involved in liver metastasis by regulating another intracellular signal transduction, and that the mechanism of ROS-triggered signal transduction has relation to tumor invasion, but not tumor metastasis in colorectal cancer **(Figure 5)**. In fact, with respect to factors other than TP, several previous reports have demonstrated that ROS generation may be induced intracellularly, in either an NADPH oxidase-dependent or a mitochondria- dependent manner, by growth factors and cytokines (TGFbeta1 and HGF) and tumor promoters (such as TPA) capable of triggering cell adhesion, EMT and migration (25, 27, 28).

In conclusion, it may be possible to prevent tumor progression by regulating ROS, which enhance cell proliferation and apoptosis suppression. For example, one possible therapeutic strategy targeting ROS-triggered signal transduction would be to increase ROS scavenging, thereby dampening H_2O_2 signaling and depressing tumor growth. In general, clarification of the true role of ROS in various cancers will lead to new strategies for cancer prevention and therapy.

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CASE REPORT

Acute Deterioration of Idiopathic Portal Hypertension Requiring Living Donor Liver Transplantation: A Case Report

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Abstract Case reports of severe idiopathic portal hypertension (IPH) requiring liver transplantation are very rare. We report the case of a 65-year-old woman who was diagnosed as having IPH. At the age of 60 years, her initial symptom was hematemesis, due to ruptured esophageal varices. Computed tomography of the abdomen showed splenomegaly and a small amount of ascites, without liver cirrhosis. She was diagnosed as having IPH and followed-up as an outpatient. Five years later, she developed symptoms of a common cold and rapidly progressive abdominal distension. She was found to have severe liver atrophy, liver dysfunction, and massive ascites. Living donor liver transplantation was then performed, and her postoperative course was uneventful. Histopathological findings of the explanted liver showed collapse and stenosis of the peripheral portal vein. The areas of liver parenchyma were narrow, while the portal tracts and central veins were approximate one another, leading to a diagnosis of IPH. There was no liver cirrhosis. The natural history of refractory IPH could be observed in this case. Patients with end-stage liver failure due to severe IPH can be treated by liver transplantation.

Keywords Idiopathic portal hypertension · Liver transplantation

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Introduction

Idiopathic portal hypertension (IPH) is a relatively rare disease, and it has been reported mostly in patients from Japan [1]). Most patients with IPH have a good prognosis with treatment for their esophagogastric varices, but some develop end-stage liver failure despite various medical treatments [2–11]. Such end-stage liver failure is an indication for liver transplantation, but details of such cases have not been fully reported in the literature. We treated a patient with severe IPH who required living donor liver transplantation (LDLT); this case allows one to observe the natural history of severe refractory IPH.

Case Report

A 65-year-old woman who had been followed-up for IPH at our hospital developed abdominal distension. At the age of 60 years, the patient presented with sudden hematemesis and was taken to a nearby hospital. On emergent upper gastrointestinal endoscopy, the ruptured esophageal varices were successfully ligated. Subsequently, the patient was transferred to our hospital.

On her admission, her vital signs (heart rate, blood pressure, respiratory rate, and body temperature) were stable. On physical examination, her abdomen was soft, flat, and not tender. Her spleen was palpable, but her liver was not. She did not have encephalopathy. The patient denied any history of blood transplantation, alcohol abuse, or medications. Laboratory data showed pancytopenia (hemoglobin 9.4 g/dl; platelet count $4.5 \times 10^4/\mu l$, and white blood cell count 1,300 /μl). Results of liver and renal function tests and electrolytes were normal. Her prothrombin time was slightly prolonged (73%). Hepatitis B

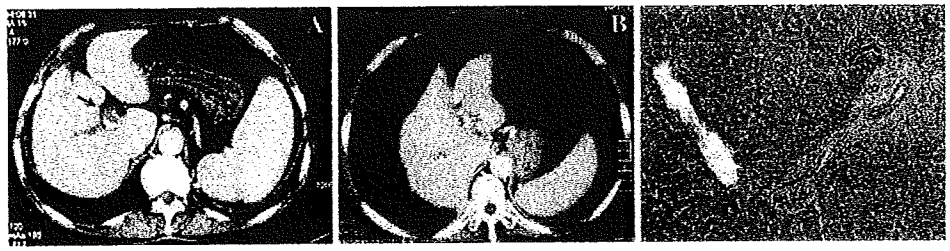

Fig. 1 (a) Computed tomography at the time of the first admission shows splenomegaly and incomplete extrahepatic portal vein thrombus (*arrow*). No liver cirrhosis or liver tumor is seen. (b) Computed tomography 5 years after the first admission shows massive ascites and a very atrophic liver. (c) Histopathological findings show collapse of the peripheral portal vein. Inflammatory cells and dilated abnormal vessels are seen near the portal tract. Hematoxylin and eosin

surface antigen, hepatitis C virus antibody, and anti-mitochondria antibody were all negative.

Computed tomography of the abdomen showed splenomegaly, incomplete extrahepatic portal vein thrombus, and a slight volume of free peritoneal fluid, but no hepatomegaly, liver cirrhosis, or liver tumors (Fig. 1a). The gastrointestinal tract, gallbladder, pancreas, kidneys, and genital organs were unremarkable. There was no obstruction of the extrahepatic portal vein or the inferior vena cava. Upper gastrointestinal endoscopy showed persistent esophageal varices.

The patient had portal hypertension that consisted of esophageal varices, splenomegaly, and pancytopenia. Laboratory data showed that liver function was completely normal, and liver cirrhosis was not found on imaging, though liver biopsy was not performed. Obstruction of the extrahepatic portal vein and the inferior vena cava, hematological malignancies, and other known diseases were ruled out. IPH was diagnosed, and the patient was followed routinely at our hospital as an outpatient, during which time she was prescribed propranolol and warfarin. Her condition remained quite stable and uneventful for 5 years.

At the age of 65 years, she developed symptoms of the common cold and rapidly progressive abdominal distension. At that time, laboratory data showed anemia (hemoglobin 7.5 g/dl), but normal platelet and white cell counts. Serum aspartate aminotransferase, alanine aminotransferase, and total bilirubin levels were normal. Her serum albumin level had decreased to 2.6 g/dl. Her prothrombin time was 44%, which was drastically prolonged, despite vitamin K administration. She also had acute renal failure (blood urea nitrogen 61 mg/dl; serum creatinine 3.8 mg/dl). Computed tomography showed massive ascites, and volumetry showed that her liver was atrophic, with only 44% of the volume of that 5 years earlier (Fig. 1b). Extrahepatic portal vein thrombus, which had been found 5 years earlier, had disappeared. The Child–Pugh score was 11 points, and the model of end-stage liver disease (MELD) score was 23 points. She was diagnosed as having non-reversible, end-stage liver failure, and she underwent liver transplantation.

At laparotomy, she had 15 l of free intraperitoneal fluid. The liver was extremely atrophic; the resected whole liver weight was only 620 g. An extended left lobe liver graft, weighting 400 g, was transplanted from her husband. The graft weight standard liver volume rate was 36.8%. A total splenectomy was also performed. The patient's postoperative course was uneventful, and she was discharged 50 days after surgery. Five months after transplantation, the patient developed enterocolitis caused by cytomegalovirus infection; she died from peritonitis following perforation of the small intestine.

The explanted liver at the time of the LDLT was atrophic, with a wavy surface. On histopathology, there was collapse and stenosis of the peripheral portal vein (Fig. 1c). The areas of liver parenchyma had become narrow, so that the portal tracts and central veins were approximate to one another. The entire specimen showed narrowing of the liver parenchyma, which was especially severe near the capsule. Inflammatory cells and dilated abnormal vessels were seen around the portal tracts. There were no pseudolobules or bridging folds. The histopathology findings were compatible with IPH. There was no liver cirrhosis or evidence of malignancy.

Discussion

Portal hypertension is usually caused by liver cirrhosis, but some cases of portal hypertension occur without cirrhosis. Sinusoidal portal hypertension of unknown etiology is regarded as IPH clinically. When diagnosing IPH, one must rule out liver cirrhosis, obstruction of the extrahepatic portal vein and the inferior vena cava, blood diseases, parasitic diseases, granulomatous hepatic disease, congenital liver fibrosis, chronic viral hepatitis, primary biliary cirrhosis, and other known diseases [12]. IPH has been found in 2.1–2.6% of livers at autopsy [13, 14]. IPH is associated with a

Table 1 Summary of case reports of severe IPH requiring liver transplantation (LT)

Author	Year	Number of patients	Gender	Age at LT	Diagnosis before LT	Period between manifestation and LT	Explanted liver weight	Pathological diagnosis	Prognosis
McDonald et al. [2]	1990	1	Male	47	Cirrhosis	10 years	973 g	NRH	Died (4 months)
Le Bail et al. [3]	1990	1	Male	48	Recurrent HCC	N/A	N/A	HCC	Well
					Alagille syndrome			NRH	
de Sousa et al. [4]	1991	1	Female	24	Multiple liver tumors	1 year	2,970 g	NRH	Well
								Budd–Chiari syndrome	
Elariny et al. [5]	1994	1	Female	44	Cirrhosis	3 years	1,850 g	NRH	Well
Nadir et al. [6]	1994	2	Male 1	60	Cirrhosis 2	4 years	N/A	NRH 2	Both well
			Female 1	55–64					
Bernard et al. [7]	1995	1	Male	34	Cirrhosis	14 years	1,070 g	ISC	Well
Loinaz et al. [8]	1998	4	Male 4	35	NRH 1	0.5 years	1,100 g	NRH 3	Died 2 (1 month, 3 months)
				25–41	Cirrhosis 1	2 months–1 year	110–2,400 g	Partial nodular transformation 1	Recurrence 1 (7 years)
					Nodular liver 1				
					Chronic liver disease 1				
Radomski et al. [9]	2000	4	Male 3	46	Cirrhosis 4	7.7 years	N/A	NRH 4	All well
			Female 1	39–55		4–13 years			
Dumortier et al. [10]	2001	8	Male 8	45	IPH 8	4.5 years	1,045 g	ISC 5	All well
				20–63		3–10 years	(630–1,520 g)	NRH 3	
Krasinskas et al. [11]	2005	16	Male 11	47	Cirrhosis 13	4.3 years	1,100 g	NRH 15	Died 1 (5 months)
			Female 5	31–64	Azathioprine-associated liver injury 2	1–11 years	600–1,550 g	ISC 9	Recurrence 2 (3.5 months, 7 months)
					NRH 1				
Our patient		1	Female	65	IPH	5 years	620 g	IPH	Died (5 months)
Total		40	Male 30	45.5 (mean)		4.6 years (mean)	1,150 g (mean)		
			Female 10						

HCC: hepatocellular carcinoma, *IPH*: idiopathic portal hypertension, *ISC*: incomplete septal cirrhosis, *NRH*: nodular regenerative hyperplasia, *N/A*: not available

spectrum of histological lesions, including incomplete septal cirrhosis, nodular regenerative hyperplasia, and partial nodular transformation [14–16]. The lesions show some degree of uneven fibrosis but may coexist in the same liver. In our patient, typical findings of incomplete septal cirrhosis, nodular regenerative hyperplasia, or partial nodular transformation were not seen. The pathogenesis of IPH is unclear, but IPH can occur in association with a variety of developmental, vascular, collagen vascular, or biliary tract diseases [6, 8, 11]. Renal failure with or without transplantation and the toxic effects of some drugs may be related to IPH [5, 8, 10, 11]. Common symptoms of IPH include splenomegaly, pancytopenia, gastrointestinal bleeding, ascites, and encephalopathy.

Patients with IPH can be treated by the usual approaches: (a) long-term medical therapy, along with balloon tamponade and vasoactive drugs for bleeding esophageal varices; (b) endoscopic variceal ligation and sclerotherapy for both long-term and short-term treatment, and (c) portosystemic shunt procedures (portocaval, mesocaval, or radiological) or evascularization surgery [8]. In general, IPH does not progress to liver cirrhosis or hepatocellular carcinoma. The prognosis for patients with IPH is mostly good, if the gastrointestinal bleeding can be controlled. However, some cases of IPH progress to end-stage liver failure.

In our case, the patient developed symptoms of the common cold and rapidly progressive abdominal distension. Collagen diseases and autoimmune disorders are known to be associated with IPH [10, 11]. The immune system plays a role in IPH, so infection may be one of the progressive factors for IPH. Though a decrease in hepatic blood flow is considered to relate to IPH progression, it is unclear whether a decrease in hepatic blood flow affected acute deterioration in this case [11]. Portal vein thrombus is one of the causes for decreased hepatic blood flow, but it was not found during the operation or in the explanted liver specimen.

Some patients with IPH which is resistant to all other therapies may be successfully treated by orthotopic liver transplantation (OLT). Several case reports dealing with OLT for severe IPH are summarized in Table 1 [2–11]. If these previous reports and ours are taken into account, there were 40 patients (30 men, 10 women) with a mean age at transplantation of 45.5 years (range 20–65 years). Gastrointestinal varices, splenomegaly, and ascites were commonly seen in patients with severe IPH who required OLT, but these symptoms are common in IPH (Table 2). No specific symptoms characterize severe IPH. Eleven patients were treated for varices, seven by sclerotherapy and four by endoscopic variceal ligation, and nine patients underwent pretransplantation portosystemic shunting procedures (six transjugular intrahepatic portosystemic shunts and three mesocaval shunts) (Table 3). The time between the clinical manifestations of portal hypertension and liver transplantation ranged from 2 months to 14 years, with a mean of 4.6 years. Patients underwent OLT because of rapidly progressing, life-threatening, complications of portal hypertension and liver disease and the inefficacy of surgical or radiological portosystemic shunting. Combined liver and kidney transplantation is performed in patients with associated severe renal failure [5, 8, 10]. Most patients with severe IPH described in the literature were well following OLT. Four patients died in the early posttransplantation period, due to herpes zoster encephalitis, suicide, sudden rupture of an unsuspected splenic aneurysm, and pneumonia [2, 8, 11]. Three patients showed evidence suggestive of recurrence of IPH in the graft liver. One patient developed symptoms 1 year after OLT [11]; two patients were asymptomatic, though their liver biopsy findings after OLT suggested recurrence [8, 11].

Making a pretransplantation diagnosis of IPH is difficult; 23 of the 40 patients with IPH were believed clinically to have cirrhosis, based on the clinical presentation, the

Table 2 Symptoms before liver transplantation in literatures

Symptom	Number of patients (%)
Gastrointestinal varices	33(83)
Splenomegaly	26(65)
Ascites	24(60)
Gastrointestinal bleeding	15(38)
Encephalopathy	11(28)
Cytopenia	6(15)
Hepatopulmonary syndrome	3(8)
Liver atrophy	1(3)
Retroperitoneal collateral rupture	1(3)
Pleural effusion	1(3)
Hepatomegaly	1(3)
Recurrent HCC	1(3)
Bacterial peritonitis	1(3)

HCC: hepatocellular carcinoma

Table 3 Treatments before liver transplantation in literatures

Treatment	Number of patients (%)
Sclerotherapy	7(18)
Transjugular intrahepatic portosystemic shunts	6(15)
Endoscopic variceal ligation	4(10)
Mesocaval shunt	3(8)
Splenectomy	3(8)
Tumorectomy for HCC	1(3)

HCC: hepatocellular carcinoma

pretransplantation biopsy findings, or the radiological images. The diagnosis was made only after posttransplantation examination of the explanted liver. Some patients who had died from what appeared to be liver cirrhosis clinically, had hidden IPH. In patients with symptoms of severe portal hypertension without liver dysfunction, IPH should be considered.

In conclusion, appropriate treatment can provide IPH patients with a good prognosis, but some patients with IPH develop end-stage liver failure. Some patients who are clinically diagnosed as having cryptogenic cirrhosis might have IPH. Patients with severe IPH which is resistant to other therapies may be good candidates for OLT, after which a favorable outcome can be expected.

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Original Article

Multicentric occurrence and spread of hepatocellular carcinoma in whole explanted end-stage liver

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Aim: Hepatocellular carcinoma (HCC) arising from the end stages of liver cirrhosis is a fair indication for liver transplantation (LT). To pathologically investigate the multicentric occurrence of relatively early staged HCC in cirrhosis, we studied whole explanted livers.

Methods: Fourteen explanted livers from patients undergoing living donor LT (LDLT) were examined. The stage of the HCCs was judged to be within the Milan criteria (M-C; a single HCC less than 5 cm or three HCCs less than 3 cm). Histological examination was performed using serially sectioned specimens 5–7 mm in width. Characterization of preoperatively detectable and undetectable lesions was also performed.

Results: In nine patients (64.3%), a total of 34 nodules were found after whole liver histological examination (WLHE). In five patients (31%), the results exceeded the M-C. The characteristics of undetectable HCCs included a minute (median size 6 mm), well-differentiated appearance (80%), with indistinct margins (85.3%) and without vascular invasion (94%). There was no recurrence in any patients at the time of follow up (median follow-up period, 30.1 months).

Conclusion: A multicentric occurrence of HCCs was demonstrated in cirrhotic livers with HCCs within the M-C. Undetectable HCCs in cirrhotic livers may have no impact on recurrence after LT.

Key words: hepatocellular carcinoma, liver transplantation, Milan criteria, whole explanted liver

INTRODUCTION

PATIENTS WITH HEPATOCELLULAR carcinoma (HCC) are a therapeutic challenge, since most tend to have chronic hepatitis or cirrhosis, which often develops into multicentric HCCs.[1,2] Liver transplantation (LT) is indicated as the treatment of choice in selected HCC patients.[3–6] In 1996, Mazzaferro and his colleagues proposed criteria for indications of LT for HCC, referred to as the Milan criteria (M-C).[7] The M-C consists of the following: solitary nodules < 5 cm in size or three nodules < 3 cm for multinodular HCC; no distant metastasis; and no evidence of vascular involvement. These factors are determined by preoperative hepatic imaging modalities. In order to investigate the real spread of HCC, a whole liver examination is warranted. However, most of the previously reported whole liver examinations were performed using livers obtained through autopsy (for example[8]). Moreover, in other studies in which incidental HCC was detected on the explanted liver, histological examination was performed only for those nodules deemed suspicious by macroscopic examination.[9–16] Thus, there has not been sufficient investigation of HCCs in the whole explanted liver.

In the present study, we used whole liver histological examination (WLHE) of transplantation explants. Clinically, these livers contained relatively early stage HCC within the M-C. Therefore, the precise existence of HCCs in a cirrhotic liver with early staged HCC could be determined. The detectability and characterization of preoperatively undetectable HCCs was also examined.

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METHODS

Patients

BETWEEN AUGUST 1997 and December 2006, 62 LDLTs were performed at the Nagasaki University Graduate School of Biomedical Sciences. In the early

years, we performed LDLT mostly in patients with biliary atresia with parental donors. Beginning in November 2000, however, we performed LDLT on 21 patients with cirrhotic livers who showed signs of HCC within the M-C, based on multidetector computed tomography scanning (MDCT) and magnetic resonance imaging (MRI) done within 1 month before transplant. Of these, 14 explanted livers underwent WLHE retrospectively and prospectively by remnant whole explanted liver. This study was approved by the local institutional review board, and written informed consent was obtained from all patients.

Patient characteristics

All 14 patients had liver cirrhosis classified as B or C stage by the Child–Pugh classification. The etiology in these cases was hepatitis C virus (HCV) infection in eight patients and hepatitis B virus (HBV) in six patients. There were six females and eight males, with a median age of 57 years (range, 48–61 years). The median values of α-fetoprotein (AFP) and protein-induced vitamin K antagonists II (PIVKAII) were 30.25 ng/mL (range, 0.8–806.1) and 23 μg/mL (range, 6–247). The clinical characteristics of the 14 patients are summarized in Table 1.

Liver transplantation and preoperative therapy for HCC

In all 14 patients LDLT had been performed, using a right lobe graft in 11 patients and a left lobe graft in three patients. The median follow-up period was 30.1 months (range, 0.53–48.5 months). In 11 patients (78.5%), pretreatment for HCC was performed prior to liver transplantation, which consisted of chemolipiodolization in six cases, radiofrequency ablation (RFA) or percutaneous ethanol injection therapy (PEIT) in four cases and chemolipiodolization with PEIT in one case. Based on the imaging findings, all HCCs were considered to be within the M-C.

Table 1 The clinical characteristics of 14 patients with cirrhosis and hepatocellular carcinoma

Age (year) (median, range)	57 (48–61)
Sex (male/female)	8/6
Cause of cirrhosis	
HBV	6
HCV	8
Child–Pugh classification	
B	5
C	9
α-fetoprotein (< 10/10–100/> 100 ng/mL)	3/7/3
PIVKAII (< 40/40 > μg/mL)	8/6
Pretransplantation treatment	
Chemolipiodolization	6
PEIT or RFA	4
PEIT + Chemolipiodolization	1
none	3

HBV, hepatitis B virus; HCV, hepatitis C virus; PEIT, percutaneous ethanol injection therapy; PIVKAII, protein-induced vitamin K antagonists II; RFA, radiofrequency ablation.

Whole liver histological examination

After explantation, the cirrhotic livers were fixed in formalin for 48 hours. The livers were then sectioned at 5–7 mm intervals, and each section was carefully inspected and mapped. All sections were embedded in paraffin, and all slides were made from the paraffin-embedded material and routinely stained with hematoxylin and eosin. The median total number of slides for each patient was 116.5 (range, 64–185 slides). All slides were examined by an experienced pathologist (co-author S. O.). The pathological diagnoses and analyses were made according to the fourth edition of *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer*, published by the Liver Cancer Study Group of Japan (LCSGJ).

Statistical analysis

A statistical comparison of categorical variables was performed using the Mann–Whitney *U*-test and the χ^2-test. Results were considered statistically significant when the *P* values were less than 0.05.

RESULTS

AT THE TIME of LDLT, there was no evidence of extrahepatic cancer spread in any of the patients. Preoperative imaging findings showed four patients with solitary HCC, five patients with double HCCs, one patient with triple HCCs and four patients with no viable HCCs. In three patients, viable HCCs had completely disappeared by the time of the preoperative treatment. Eight patients had a local recurrence or another new lesion in the liver based on imaging before LDLT. All patients met the M-C with a solitary nodule < 5 cm in size or three nodules < 3 cm for multinodular HCC.

Detection of HCCs by WLHE

In nine patients (64.3%), undetectable nodules were found after WLHE, and four patients (28.6%) had preoperatively detectable nodules but no new lesions (Fig. 1). In nine cases, small HCCs that could not be

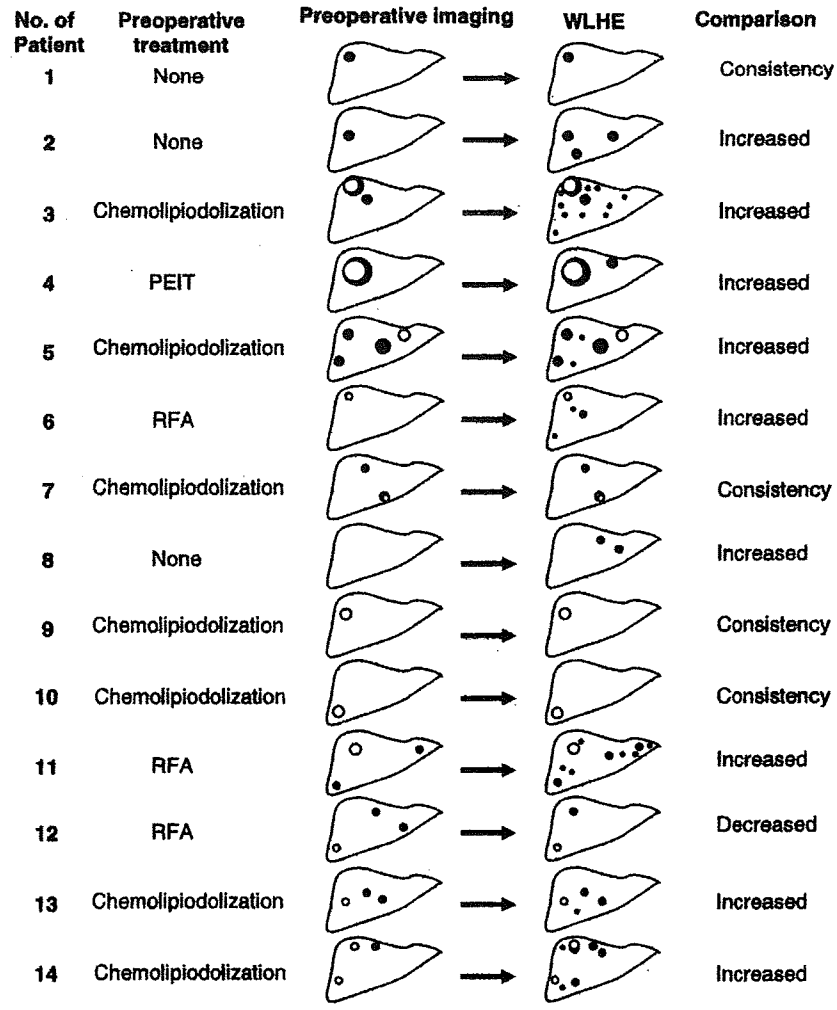

Figure 1 Detection of hepatocellular carcinoma using preoperative imaging and postoperative whole liver histological examination.

● Viable hepatocellular carcinoma (HCC).
○ Preoperative treatment with no viable HCC.
PEIT, percutaneous ethanol injection therapy; RFA, radiofrequency ablation; WLHE, whole liver histological exmination.

detected by the current imaging modalities were identified only by pathological examination. One patient (7.1%) (case 12) had a decreased number of HCCs compared to the number determined from preoperative imaging. The distribution of preoperatively undetectable nodules, which was based on segmental anatomy of the liver, was as follows: four nodules in segment 2, four nodules in segment 3, seven nodules in segment 4, one nodule in segment 5, six nodules in segment 6, three nodules in segment 7 and nine nodules in segment 8 (Fig. 1). After WLHE, five out of the 14 patients (35.7%) were beyond the M-C. When we compared the results for the number and largest size of HCCs between imaging and WLHE, the largest size of HCCs was not altered by WLHE, but the number of HCCs was increased by WLHE (Fig. 2). This was because small HCCs that could not be preoperatively detected by imaging were found in the cirrhotic liver by whole liver investigation.

Characteristics of preoperatively detectable HCCs

A total of 49 nodules were found by WLHE (Table 2). Fifteen nodules were found through preoperative images taken after histological examination, but two

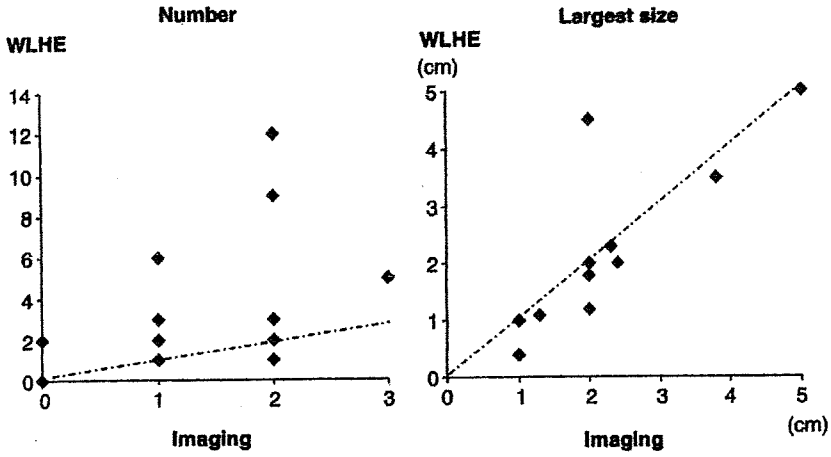

Figure 2 The relationship of size and number between imaging and whole liver histological examination.

nodules detected preoperatively were not found in the explanted liver after WLHE. The median diameter of the 15 preoperatively detectable HCCs was 18 mm (range, 10–50 mm). There were seven well-differentiated HCCs (46.7%) and eight moderately differentiated HCCs (53.3%). The preoperatively detectable nodules showed expansive growth (11/15; 73.3%) with fibrous capsules (60%) (Table 2). Only two nodules (13.3%) showed microscopic vascular invasion surrounding the main tumor in the detectable HCCs.

Characteristics of preoperatively undetectable HCCs

Thirty-four HCCs that were undetectable preoperatively were found by WLHE (Table 2). The median diameter of the nodules was 6 mm (range, 2–15 mm). The undetectable nodules consisted of 25 well-differentiated HCCs (73.5%), nine moderately differentiated HCCs (26.5%) and no poorly differentiated HCCs. The characteristic features of the preoperatively undetectable nodules included infiltrative growth (29/34; 85.3%), absence of fibrous capsules (32/34; 94.1%) and absence of microscopic vascular invasion (32/34; 94.1%).

Pathological comparison of preoperatively undetectable and detectable HCCs

By comparing the pathological features of undetectable and detectable HCCs based on the preoperative imaging results, we examined the relationship between tumor size and each of tumor differentiation, tumor growth and the presence of fibrous capsules (Figs 3–5). No significant difference in differentiation or growth was found between the preoperatively detectable and undetectable tumor size. However, we found significant differences in the presence of fibrous capsules between the undetectable and detectable HCCs (Fig. 4).

Table 2 The characteristics of hepatocellular carcinoma on preoperative imaging study and postoperative histological study

	HCC on imaging ($n=15$)	HCC only on WLHE ($n=34$)	
Diameter (median, range) (mm)	18 (10–50)	6 (2–15)	$P=0.0000006$
Differentiation			
Well	7 (46.7%)	25 (73.5%)	NS
Moderate	8 (53.3%)	9 (26.5%)	NS
Poor	0	0	
Fibrous capsule	9 (60.0%)	2 (5.9%)	$P=0.000028$
Growth			
Expansive growth	11 (73.3%)	5 (14.7%)	NS
Infiltrative growth	4 (26.7%)	29 (85.3%)	NS
Microvascular invasion	2 (13.3%)	2 (5.9%)	NS

HCC, hepatocellular carcinoma; NS, not significant; WLHE, whole liver histological examination.

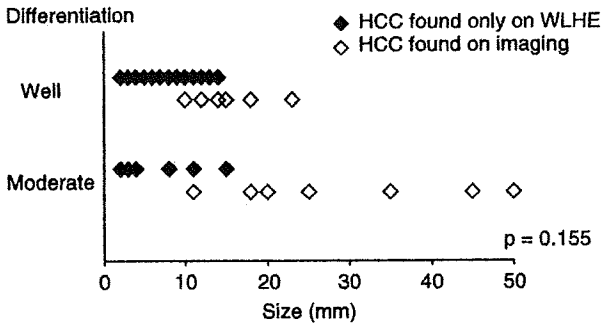

Figure 3 The relationship between differentiation and size between imaging and whole liver histological examination.

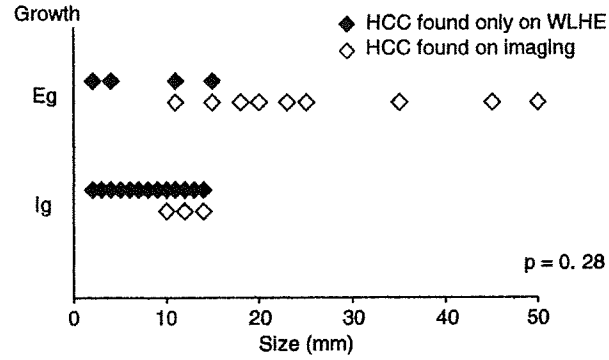

Figure 5 The relationship between growth and size between imaging and whole liver histological examination.
Eg, expansive growth; Ig, infiltrative growth.

Recurrence of HCCs

In the follow-up period (median follow-up, 30.1 months), there was no recurrence in any patient after LDLT.

DISCUSSION

IN THIS STUDY, we have demonstrated a discrepancy in the number of HCCs determined from preoperative imaging studies and the number determined from histological measurements using WLHE. It is especially important to note that multicentric HCCs were diffusely present in the end-stage cirrhotic liver, which is clinically significant since local therapy for only those HCCs that are detectable might not be a practical curative procedure in such cirrhotic livers. In addition, this study demonstrated that even HCCs within the M-C, which are usually regarded as early HCCs, can coincide with much earlier HCCs in the severely cirrhotic liver. This finding has not been demonstrated in autopsied livers from patients who died of advanced HCC.

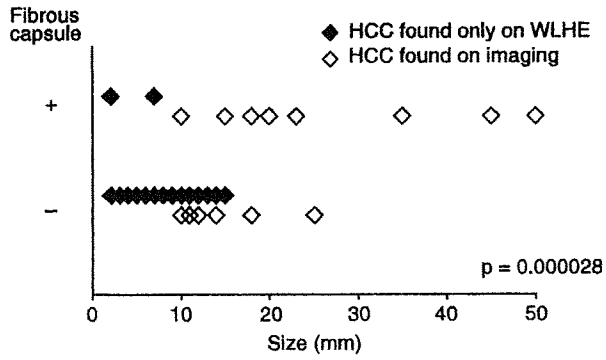

Figure 4 The relationship between fibrous capsule and size between imaging and whole liver histological examination.

In the present study, the accuracy of preoperative diagnosis for HCC was 28.6%, which is rather low compared to other reports.[9–16] However, other studies used histological examination only for those nodules that were suspicious based on imaging findings, and not for the whole liver. Moreover, MDCT tends to overdiagnose HCC, with a false-positive diagnosis rate as high as 8–11.8%.[11,12] In addition, in a previous study MDCT could detect only 61% of lesions smaller than 2 cm and 93.6% of lesions 2 cm or larger.[12] Since our study demonstrated that the median diameter of preoperatively undetectable nodules was 6 mm (range, 2–15 mm), the overlooked lesions in previous studies were definitely below the detectable range of CT.

Of the 14 patients preoperatively within the M-C, undetectable HCCs were found in nine patients (64.3%), with the result that five patients (35.7%) exceeded the M-C after WLHE, mainly due to the increased number of HCCs. The diameters of the largest HCCs determined from WLHE were identical to preoperative measurements. Therefore, current imaging modalities, such as MDCT, are good at evaluating the size of viable large HCCs, but not for determining the number of small HCCs. The reason the small HCCs were not detected by the current imaging modalities might be that they were characterized by an absence of fibrous capsules and an unclear border between the cancerous lesions and the normal liver tissue. Another possible reason for the failure of the imaging methods to detect small HCC might be that the blood supply to the early HCC came more from the portal vein than the hepatic artery, as in the case of advanced HCC. The

distribution of undetectable HCCs did not show any special features; that is, undetectable HCCs could be found in any segment in the cirrhotic liver, implying that MDCT did not have blind areas.

In this study, there was no recurrence of HCCs after LT during the median follow-up period of 30.1 months, although two patients showed vascular invasion in the explanted livers by microscopic examination. Thus, HCCs in cases of advanced cirrhotic liver could be a multicentric phenomenon, and undetectable HCCs might not affect the survival rate after LT. Therefore, in the era of MDCT, the M-C would seem to be too strict a set of criteria for determining whether or not LT is indicated.[17–20] In other words, if we do follow the M-C as an indication of LT for HCC, the recurrence rate of HCC should be minimal.

In conclusion, HCCs in the severely cirrhotic liver might be characterized by multicentric occurrence. In this study, there were no recurrent HCCs after LDLT. Thus, preoperatively undetectable HCCs might not be associated with recurrence of HCC after LDLT. However, a high rate of recurrence can be expected when local therapy is performed for HCC, even with early-stage HCC, as determined by the M-C.

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SURGERY TODAY

How to Do It

A Secure Taping Technique for a Liver Hanging Maneuver Using a Surgical Probe

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Abstract
A liver hanging maneuver is currently being applied for various types of hepatectomies. The most difficult and important step of this technique is to encircle the liver with tape that is passed between the liver and the inferior vena cava, using a blind dissection. This report describes a secure technique for taping utilizing a surgical probe.

Key words Hepatectomy · Hanging maneuver

The liver hanging maneuver was originally introduced as a safe procedure to use during a right hepatectomy,[1] and in a modified form it is now being applied for various types of hepatectomies and liver transplantations.[2–5] When this technique is applied during a right hepatectomy or a left hepatectomy with the caudate lobe, the most difficult and important step is to encircle the liver with tape that is passed between the liver and the inferior vena cava (IVC), using a blind dissection. Several studies have so far been proposed using a lightly curved Kelly clamp during this step, with acceptable results,[6,7] but IVC injury is always a great concern during this step.[8] Some technical inventions have also been introduced to avoid this significant injury, such as an ultrasonic- or endoscopic-assisted dissection between the liver and IVC.[6,9] This report describes a secure technique for taping which utilizes a surgical probe.

During the dissection of the cranial part of the liver, the space between the right hepatic vein (RHV) and the middle hepatic vein (MHV) is carefully exposed. After a subsequent cholecystectomy and hilar dissection, the hepatoduodenal ligament is encircled with the tape and then the ligament is lifted to allow a fine view of the caudal edge of the liver. The thin part of the caudate lobe is dissected away from the IVC and the surgical probe is carefully inserted in front of the IVC with or without ultrasound guidance to avoid significant short hepatic vein injury. After the tip of the surgical probe is identified at the space of the cranial end between the RHV and MHV, the probe is advanced while feeling the tip and bending it ventrally with the finger (Fig. 1). After the probe is fully inserted with a sufficient length above the space between the RHV and MHV (around 5 cm or longer), a 3-0 monofilament suture is tied at the tip of the probe and the tape is tied at the opposite end of the suture. The liver is finally encircled with the tape by pulling the probe down carefully (Fig. 2). Thus far, this procedure has been employed in four living donor hepatectomies of the left liver with the caudate lobe and in three right hepatectomies for a huge hepatocellular carcinoma. In all cases, the taping was easily accomplished without any problems.

The liver hanging maneuver is a favorable procedure in various types of hepatectomies. However, a blind dissection between the liver and IVC is still a challenging procedure, with the risk of significant bleeding due to IVC injury. Several studies have described an ordinary procedure with a lightly curved Kelly clamp with acceptable results, but bleeding complications occurred.[7] Among the seven living donor hepatectomies so far performed with a blind dissection using a Kelly clamp, one patient had significant bleeding (3150 g) due to an IVC injury even under assistance with ultrasound. Based on this experience, a safer and more comfortable technique was therefore developed using a surgical probe.

The surgical probe is a conventional device found in any center and it has several advantages over the Kelly clamp. First, it is thin and light but the tip is blunt, so that the surgeon can easily feel resistance when it

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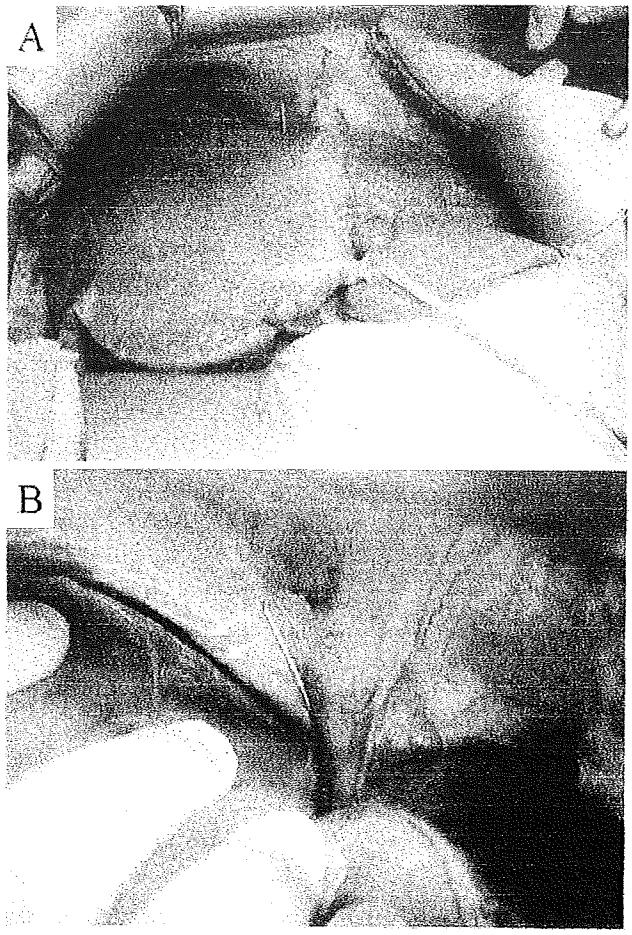

Fig. 1. **A** Insertion of the surgical probe between the liver and inferior vena cava. **B** The probe is inserted while bending it ventrally (*arrow*)

advances the wrong way and significant injury even of small short hepatic veins can thus be avoided. Second, the probe is relatively coarse but flexible, so that it can be used while bending it ventrally, as shown in this study. With this maneuver, tape is easily attached with the surgical probe, interposed with a 3–0 monofilament suture. The feasibility of this technique should be determined by a prospective and randomized study. This simple technique to encircle the liver is easy to perform and it is recommended as a secure technique to use when carrying out a liver hanging maneuver.

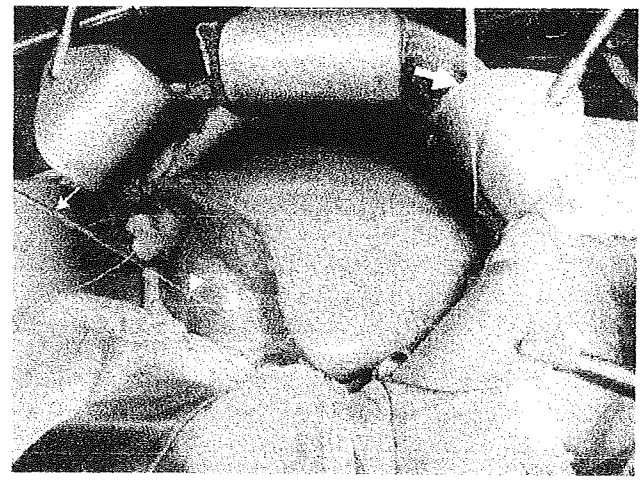

Fig. 2. Tape (*large arrow*) is attached to the surgical probe (*small arrow*), interposed with a 3-0 monofilament suture (*arrowhead*)

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Metabolism for cyclosporin A during liver regeneration after partial hepatectomy in rats

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Abstract

AIM: To elucidate the metabolism and the effect of the cyclosporin A (CyA) as a representative immunosuppressive drug used in transplantation in a partially hepatectomized rat model.

METHODS: CyA was administered to rats that underwent a 70% hepatectomy. These rats were randomly assigned into three groups according to the dose of CyA administration as follows; (group 1) water, (group 2) 5 mg/kg CyA, (group 3) 10 mg/kg CyA. On postoperative days-1, 3, 7 and 14, the rats were killed to analyze the serum concentration of CyA, the liver regeneration ratio, biochemical or histological markers, and mRNA expression using reverse transcriptase-polymerase chain reaction method to determine albumin and cytochrome p450 expression.

RESULTS: The serum concentration of CyA in group 3 was significantly higher than group 2 during liver regeneration. CyA enhanced the liver regeneration in a dose dependent manner. The mRNA expression associated with CyA metabolism was significantly decreased on day 14, while preserving the albumin producing activity.

CONCLUSION: These data indicate that the p-450 activity required to metabolize the CyA may be reduced during regeneration of the remnant liver after a hepatectomy, which may, therefore, be linked to difficulty in controlling the optimal dose of CyA during early period of LDLT.

Key words: Cyclosporin A; Liver regeneration; Partial hepatectomy; Rat

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INTRODUCTION

Orthotopic liver transplantation is an established treatment for patients with end-stage liver disease. However, donor organ shortages remain extremely problematic. To address this issue, living donor liver transplantation (LDLT) was developed[1]. During transplantation, the liver graft is subjected to a variety of potential hepatic injuries including ischemic injury associated with organ harvesting and the obligate storage before revascularization, reperfusion injury following revascularization, immunological attack caused by the immune system of the recipient, toxicity of certain drugs used during the post-transplant period, and certain infections[2,3]. After transplantation the liver graft goes into a regeneration process, which may be important for the overall success of the transplant procedures. Notably, the liver graft must be capable of normal growth, repair, and regeneration in the presence of immunosuppressive drugs such as calcineurin inhibitors. The aim of the present study was, therefore, to investigate the pharmacokinetics of cyclosporin A (CyA)[4] and its effect on liver regeneration and metabolic

activity to elucidate the mechanism of metabolic activity and serum concentration of cyclosporin A as an example of calcineurin inhibitors administered during liver regeneration in a rat model.

MATERIALS AND METHODS

Animals and treatments

Adult male Sprague Dawley rats, weighting 250-320 g (CRJ Charles River Japan, Kanagawa, Japan), were provided with water, and a standard laboratory diet ad libitum. All of the studies were performed according to the rules and regulations of the University of Nagasaki Research Animal Resources Guidelines.

Surgical procedures

A 70% hepatectomy was carried out according to the method described by Higgins and Anderson[5] under light ether anesthesia. Surgery was performed between 9:00 and 12:00 a.m. to avoid diurnal variation in the regenerative responses. The rats were randomly assigned to three groups, and treated daily by gavage beginning immediately after the hepatectomy. Group 1 animals were given water. Group 2 animals received 5 mg/kg CyA (Neoral®, Novartis Pharma, Basel) and group 3 animals received 5 or 10 mg/kg CyA. These CyA doses were selected based on the results reported by Morii *et al*[6].

In each group, five rats were killed before and at day 1, 3, 7 and 14 after the hepatectomy. Immediately before they were sacrifices, blood samples were obtained from the inferior vena cava. The remnant liver was removed to investigate hepatic restoration. The experimental protocol is demonstrated in Figure 1.

Serum concentration of CyA

The serum concentration of CyA was measured in the whole blood by fluorescence polarization according to the manufacturer's protocols (AxSYM® analyzer, Abbott, Tokyo)[7].

Regeneration ratio

The liver regeneration ratio in each experiment was defined as the ratio of the remaining liver weight to the initial body weight.

Serum ALT and T-Bil level

To evaluate liver toxicity of CyA administration, plasma concentrations of alanine aminotransferase (ALT) and total bilirubin (T-Bil) were examined using an automated analyzing system according to the manufacturer's protocol.

RT-PCR analysis

Total hepatic RNA was prepared by the method as described previously[8] and used for the determination of the expression levels of albumin (ALB) and cytochrome-P 3A2 (CYP3A2). In addition, the level of gene expression of glyceral-dehyde-3-phosphate-dehydrogenase (GAPDH) was measured as an internal control. Complementary DNA (c-DNA) was prepared from total RNA by the method described previously[8]. The primers used in the present study are listed in Table 1.

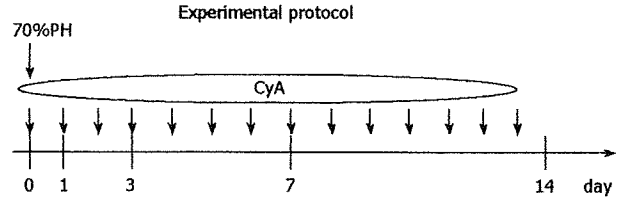

Figure 1 Administration schedule of CyA. Rats underwent a 70% hepatectomy immediately followed by the daily administration of CyA for up to 14 d per os. Blood samples were collected on post operative day 1, 3, 7 and 14.

Statistical analysis

The data are expressed as the mean ± SD (n = 5). Statistical analyses were performed by unpaired, two tailed Student's t-test. A P value less than 0.05 was considered to be significant.

RESULTS

Changes of serum concentration of CyA during liver regeneration

Figure 2 shows that the concentration of CyA reached a maximum during 3 to 7 d, and gradually declined thereafter. The levels of CyA in the PH group were significantly higher than that in control group.

The effect of CyA on liver regeneration ratio

As shown in Figure 3, the lower concentration of CyA (5 mg) did not affect the liver regeneration potential during the observation period; however, the rate of liver regeneration was significantly higher than that in the low CyA group on postoperative day 7.

Changes of hepatocyte specific gene expression during liver regeneration

Alb mRNA expression remained constant during liver regeneration, while hepatocyte specific p450 activity-CYP3A2 was significantly reduced on postoperative day 14 (Figure 4).

The effect of CyA on liver function

Rats were anesthetized and blood samples were collected through the tail vein at the indicated time points. ALT and T-Bil levels were measured as indicators of liver function. On day 1, plasma ALT concentrations increased during the first 24 h after the hepatectomy and then decreased gradually returning to the preoperative values at 72 h. There was no significant difference between the groups (Figure 5).

As shown in Figure 5, the ALT level in control animals were slightly increased, and thereafter gradually reduced. There was no statistically significant difference in any of the groups.