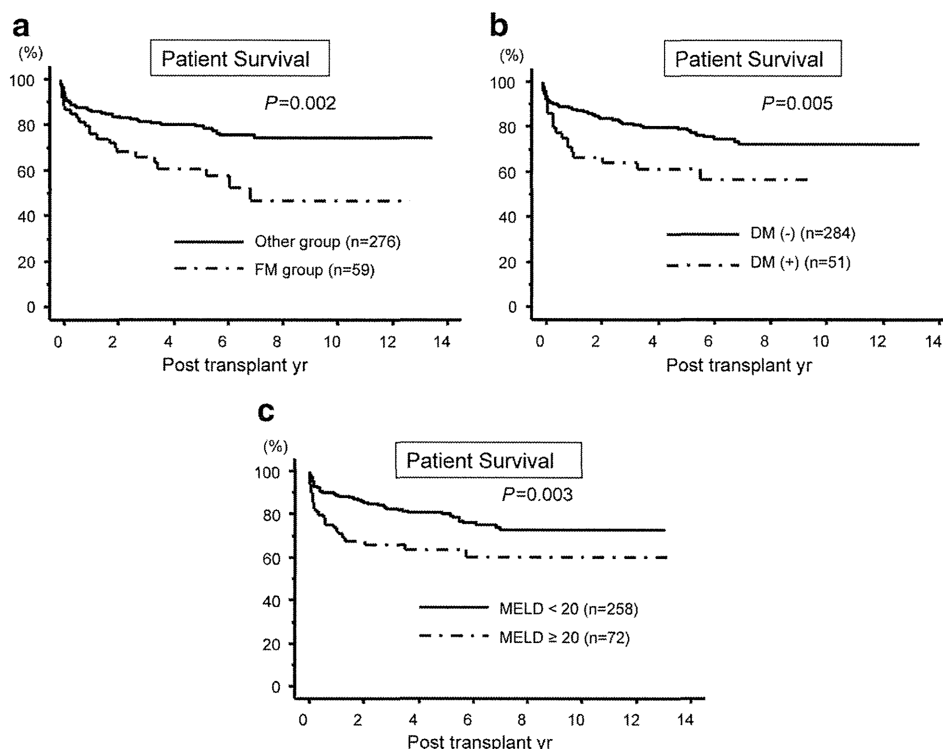

FIGURE 2. (a) Patient survival after LDLT between the two groups according to gender combination. The 1-, 3-, 5-, and 10-year patient survival rates in the FM group were 80.6%, 66.8%, 61.8%, and 47.7%, respectively, and those in the other combination groups were 86.7%, 81.3%, 79.5%, and 74.5%, respectively. The FM group had significantly worse patient survival rates compared with the other combination group (P=0.002). (b) Patient survival after LDLT between the two groups according to pretransplant DM. The 1-, 3-, 5-, and 10-year patient survival rates in the DM (+) group were 70.9%, 63.9%, 61.0%, and 56.6%, respectively. Those in the DM (−) group were 88.3%, 81.3%, 79.0%, and 72.3%, respectively. The DM (+) group had significantly worse patient survival rates compared with the DM (−) group (P=0.005). (c) Patient survival after LDLT between the two groups according to pretransplant MELD. The 1-, 3-, 5-, and 10-year patient survival rates in the MELD score more than or equal to 20 group were 72.2%, 65.7%, 63.3%, and 60.1%, respectively. Those in the MELD score less than 20 group were 89.1%, 82.4%, 80.0%, and 72.7%, respectively. The MELD score more than or equal to 20 group had significantly worse patient and graft survival rates compared with the MELD score less than 20 group (P=0.003). LDLT, living donor liver transplantation; FM, female donor to male recipient; DM, diabetes mellitus; MELD, model for end-stage liver disease.

sis (41/4), hepatitis B (10/18), cryptogenic (6/11), PSC (2/9), alcohol abuse (3/9), biliary atresia (4/2), and others (15/4) (Table 1).

Donor and Graft Selection

Donors were selected from among candidates who hoped to be living donors (*11, 12*). Consequently, 335 donors (111 women and 224 men) were enrolled. The relationships between donors and recipients were as follows: son (n=141), daughter (n=47), brother (n=36), wife (n=25), sister (n=20), husband (n=21), mother (n=10), father (n=10), nephew (n=7), son-in-law (n=3), cousin (n=2), father-in-law (n=2), and others (n=11). The graft types included left lobe with caudate lobe graft (n=194), right lobe graft without the MHV (n=117), right lobe graft with MHV (n=3), left lobe graft (n=16), and posterior segment graft (n=5). Donors were required to be spouses or within the third degree of consanguinity with recipients and to be between 20 and 65 years of age. For a donor who was not within the third degree of consanguinity, individual approval was obtained from the ethics committee of Kyushu University Hospital. Good Samaritan organ donations were not used.

We used three-dimensional computed tomography for volumetric analysis and delineation of vascular anatomy. The SLW of recipients was calculated according to the formula of Urata (*11, 12*). GW was predicted by computed tomographic volumetric analysis. The decision about graft type for the recipients was based on the preoperatively predicted GW to SLW (GW-SLW) ratio. A left lobe graft was used when the preoperatively predicted GW-SLW ratio was more than 35%.

Postoperative Management

Graft harvesting technique, recipient surgery, and perioperative management of the recipients, including immunosuppression regimens, have been previously described (*11, 12, 21*). Bile ducts were reconstructed using the Roux-en-Y (n=81) or duct-to-duct (n=251) techniques. Bile ducts were not reconstructed in two cases because of intraoperative bleeding. We initiated immunosuppression with a protocol based on tacrolimus (Prograf; Astellas Pharma Inc., Tokyo, Japan) or cyclosporine A (Neoral; Novartis Pharma K.K., Tokyo, Japan).

All patients had monthly follow-ups, and the median follow-up period was 1377 days, with 369 days and 2186 days as the 25th and 75th percentiles, respectively. Patient survival was defined as the time period between LDLT and patient death.

Impact of Recipient and Donor Gender

The 335 patients and donors were classified into four groups according to the donor-recipient gender combinations as follows: MM group (n=104), MF group (n=120), FM group (n=59), and FF group (n=52). The 1-, 3-, 5-, and 10-year patient and graft survival rates were compared among the

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groups. Univariable and multivariable analyses were performed to identify the factors associated with patient mortality after the LDLT.

Statistical Analysis

The significance of differences among four groups was determined by one-factor analysis of variance. Cox regression analysis was applied to the univariable and multivariable analyses. Survival was calculated by the Kaplan-Meier product-limited method, and differences in survival between two groups or among all four groups were then compared using the log-rank test. Data were expressed as mean±standard deviation. All statistical analyses were performed using StatView 5.0 software (SAS Institute, Inc., Cary, NC). A P value of less than 0.05 was considered significant.

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ORIGINAL ARTICLE

Effects of a whey peptide-based enteral formula diet on liver dysfunction following living donor liver transplantation

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Abstract
Background and aims Whey protein, a protein complex derived from milk is well known as a functional food with a number of health benefits. MEIN® (Meiji Dairies Co., Tokyo Japan) is a functional liquid-type nutritional diet containing whey-hydrolyzed peptide. In this study, we examined the effects of MEIN® on postoperative liver dysfunction in patients who underwent living donor-related liver transplantation (LDLT).
Methods Sixteen adult patients transplanted between 2005 and 2011 at our institute were evaluated retrospectively. In MEIN group ($n = 8$), administration of MEIN® was started around 14 days after liver transplantation when serum liver enzymes were re-elevated, while MEIN® was not administered in the control group ($n = 8$) who did not have postoperative liver dysfunction.
Results In the preoperative clinical characteristics, the model for end-stage liver disease score in the MEIN group was significantly lower than that in the control group. The graft-to-recipient body weight ratio in the MEIN group was lower than that in the control group. Elevation of enzymes in the liver function tests such as alanine aminotransferase and total bilirubin, and C-reactive protein in the MEIN group had significantly improved, and became almost normal values which were the same as those in the control group.
Conclusion These findings suggest that administration of whey-hydrolyzed peptide attenuates the post-transplant liver dysfunction and may avoid an unnecessary liver biopsy.

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Keywords Liver transplantation · Whey peptide · Acute cellular rejection · Enteral nutrition

Abbreviations
AST Aspartate aminotransferase
ALT Alanine aminotransferase
CRP C-reactive protein
CT Computed tomography
GRWR Graft-to-recipient body weight ratio
HBV Hepatitis B virus
HCV Hepatitis C virus
LDLT Living related donor liver transplantation
LPS Lipopolysaccharide
MRCP Magnetic resonance imaging
MELD Model for end-stage liver disease
T-Bil Total bilirubin

Introduction

After liver transplantation, the levels of liver enzymes, such as aspartate aminotransferase (AST), and alanine aminotransferase (ALT), are often elevated due to acute cellular rejection, the recurrence of virus hepatitis, portal vein thrombosis, hepatic artery thrombosis, hepatic vein obstruction, bile duct complications, drug-induced liver injury, and various types of infection [1, 2]. The presence of vessel thrombosis or obstruction and bile duct complications can be determined by imaging modalities, such as ultrasonography (US), dynamic computed tomography

(CT) or magnetic resonance imaging (MRI). In the patients with rejection or recurrence of hepatitis, a liver biopsy may be required [3, 4]; however, there may be some serious risks associated with such biopsies, such as bleeding, bile leakage or other organ injury. When the etiology of the elevation of liver enzymes can be determined, the liver biopsy may be avoidable [5–8].

Careful perioperative management, including defined nutrition, should be considered for patients undergoing liver transplantation [9]. Several studies have shown that immune-modulating nutritional formulas may have a role in improving the preoperative nutritional status, hastening recovery after transplantation, and reducing postoperative infectious complications [10]. Therefore, we retrospectively evaluated the effects of immune-modulating formulas in recipients after living donor-related liver transplantation (LDLT). In this study, we used a whey-hydrolyzed peptide for the formula, which is a protein complex derived from milk. It has been reported to have antioxidant, antihypertensive, antitumor, antiviral, hypolipidemic, and antibacterial effects [11]. The whey proteins from milk include β-lactoglobulin, α-lactalbumin, glycomacropeptide, immunoglobulins, and lactoferrin, and are used as a functional food that is considered to provide a number of health benefits [11]. These proteins also have been reported to exert anti-inflammatory and hepatoprotective effects [12–15]. Whey-hydrolyzed peptide has hepatoprotective effects against hepatitis and is more easily absorbed than whey protein. A previous study showed that the serum lipid peroxide levels significantly decreased, and the interleukin (IL)-2 levels and natural killer (NK) activity significantly increased in patients with chronic hepatitis due to hepatitis B virus (HBV) and C virus (HCV) infection following consumption of whey-hydrolyzed peptide [16].

MEIN® (Meiji Dairies Co., Tokyo, Japan) contains an abundance of whey-hydrolyzed peptide, which is extracted from bovine milk. This nutritional formula, like other whey-derived proteins, has been reported to have antioxidant, antihypertensive, antitumor, antiviral, hypolipidemic, and antibacterial effects in vivo and in vitro [11, 14, 17–19]. Moreover, early enteral nutrition with MEIN® was useful to prevent post-LDLT bacteremia and shorten the postoperative hospital stay in transplant patients [20].

In the present study, we evaluated the usefulness of MEIN® including a whey-hydrolyzed peptide for patients with re-elevation of the liver enzyme levels after LDLT.

Patients and methods

Study design and enrolled patients

Eight adult patients who received transplants between 2005 and 2011 at Tokushima University Hospital were evaluated retrospectively. The indication for LDLT was HCC in three cases, HCV infection in three cases, HBV infection in one case and Wilson's disease in one case (Table 1). Eight patients who did not have postoperative liver dysfunction and did not receive the MEIN formula served as the control group.

Table 1 Patients characteristics

Background	MEIN ($n = 8$)	Control ($n = 8$)	p value
Age	49 ± 13	55 ± 3	0.21
Gender (F/M)	3/5	4/4	0.25
Indication for LDLT			
HCC	3	0	
HCV-related liver cirrhosis	3	1	
HBV-related liver cirrhosis	1	4	
Others	1	3	
Child-Pugh classification A/B or C	2/6	0/8	N.A
MELD score	10 ± 4	16 ± 6	0.04
ABO compatibility			
Identical/compatible	6	8	N.A
Incompatible	2	0	
Graft type (left lobe/right lobe)	7/1	6/2	0.41
Graft versus recipient weight (GRWR)	0.72 ± 0.12	0.89 ± 0.19	0.06

Perioperative management of LDLT

Liver transplantation was performed using a living related donor. The surgical procedures for the donor and recipient have been described previously [21]. For immunosuppressive therapy, induction consisted of two doses of basiliximab (Simulect®, NOVARTIS) on postoperative days 0 and 4. Standard immunosuppressive therapy at discharge consisted of corticosteroids and calcineurin inhibitors (either tacrolimus or cyclosporine) with mycophenolate mofetil (MMF). Prednisolone was discontinued on day 21 after the surgery. In ABO incompatible cases, we administered preoperative anti-CD20 antibodies (Rituximab®, 375 mg/m^2) and performed plasma exchange for 3 days.

MEIN® composition

A commercially available enteral nutrition, MEIN® (Meiji Dairies Corporation, Tokyo, Japan) was used in this study. It is a newly designed enteral formula, including whey peptide. In terms of its general composition, it has 1 kcal/ml, including 50 mg/ml of protein, 28 mg/ml of fat, 133 mg/ml of carbohydrate, 12 mg/ml of alimentary fiber, 6 mg/ml of

ash content, and is made using 84.4 g/100 ml of water. Moreover, it includes 2.25 g/100 ml of essential amino acids and 2.63 g/100 ml of nonessential amino acids. The Fischer ratio is 3.7. The protein sources used for MEIN® are whey-hydrolyzed peptide and fermented milk.

Administration of MEIN®

The administration of MEIN® was started 14.6 ± 2.4 days after liver transplantation in the patients ($n = 8$) who showed a re-elevation of liver enzyme levels (MEIN group). The patients were administered MEIN® three times a day either orally or through a tube jejunostomy (Fig. 1).

Blood biochemistry

All patients were monitored for the liver enzyme levels, including AST and ALT, alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (γGTP), as well as the total bilirubin (T-Bil) and C-reactive protein (CRP) levels as parameters of liver dysfunction before the administration of MEIN, after 7 days of administration and 14 days after starting the administration of MEIN.

Statistical analysis

Statistical comparisons of the mean values were conducted using a one-way analysis of variance (ANOVA). All results are presented as the mean ± standard deviation (SD). A p value <0.05 was considered to be statistically significant. The statistical analysis was performed using the JMP® 7.0.2 statistical software program (SAS Institute, Cary, NC).

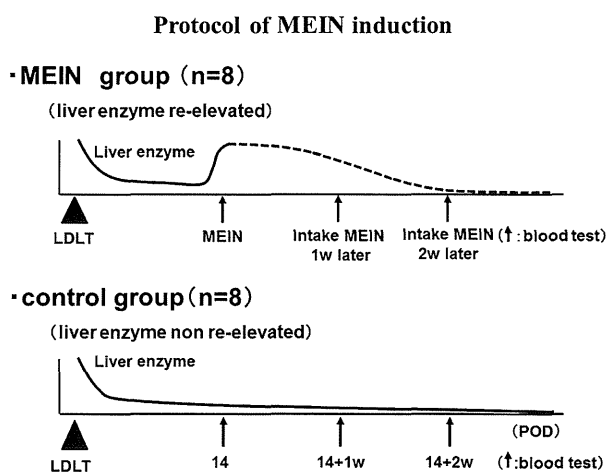

Fig. 1 The timing of the re-elevation of liver enzyme levels and the administration of MEIN

Results

Patient characteristics

The model for end-stage liver disease (MELD) score in the MEIN group was significantly lower than that in the control group (10 ± 4 vs. 16 ± 6, $p = 0.04$) (Table 1). In the control group, all of the patients categorized as having Child B/C status, while there were two Child A patients in the MEIN group. In the control group, there were no ABO incompatible cases, while there were two ABO incompatible cases in the MEIN group. The graft-to-recipient body weight ratio (GRWR) in the MEIN group was lower than that of the control group (0.72 ± 0.12 vs. 0.89 ± 0.19, $p = 0.06$). There were no significant differences in any of the other characteristics, including the patient age, gender or graft type.

Blood biochemistry

The serum levels of AST and ALT 1 and 2 weeks after starting the administration of MEIN® were significantly lower than those before MEIN® administration (AST: 101.4 ± 61.5 vs. 52.3 ± 31.4 vs. 45.8 ± 20.5, ALT: 201.1 ± 133.9 vs. 123.1 ± 104.2 vs. 79.9 ± 47.8, $p < 0.05$). The serum levels of T-Bil and CRP 2 weeks after starting the administration of MEIN® were significantly lower than those before MEIN® administration (T-Bil: 4.3 ± 4.9 vs. 2.5 ± 4.5, CRP: 1.7 ± 1.0 vs. 0.8 ± 0.7, $p < 0.05$) (Fig. 2a, b). After 2 weeks of MEIN, these values were almost identical to those values in the control group. The serum levels of ALP and γGTP did not differ significantly in the patients between before and after the administration of MEIN®.

Discussion

Patients often experience a re-elevation of liver enzyme levels around 2 weeks after LDLT, even after the early postoperative liver dysfunction is improved. In such cases, it is necessary to consider several possible etiologies, such as acute cellular rejection, recurrence of virus hepatitis, portal vein thrombosis, bile duct complication, and drug-induced liver injury, in order to optimize the treatment strategy. It is worth noting that the administration of an enteral formula (MEIN®), which contains whey-hydrolyzed peptide, significantly improved the re-elevated liver enzyme levels after LDLT in the present study. This is the first report demonstrating that whey-hydrolyzed peptide can ameliorate the liver dysfunction in patients after LDLT.

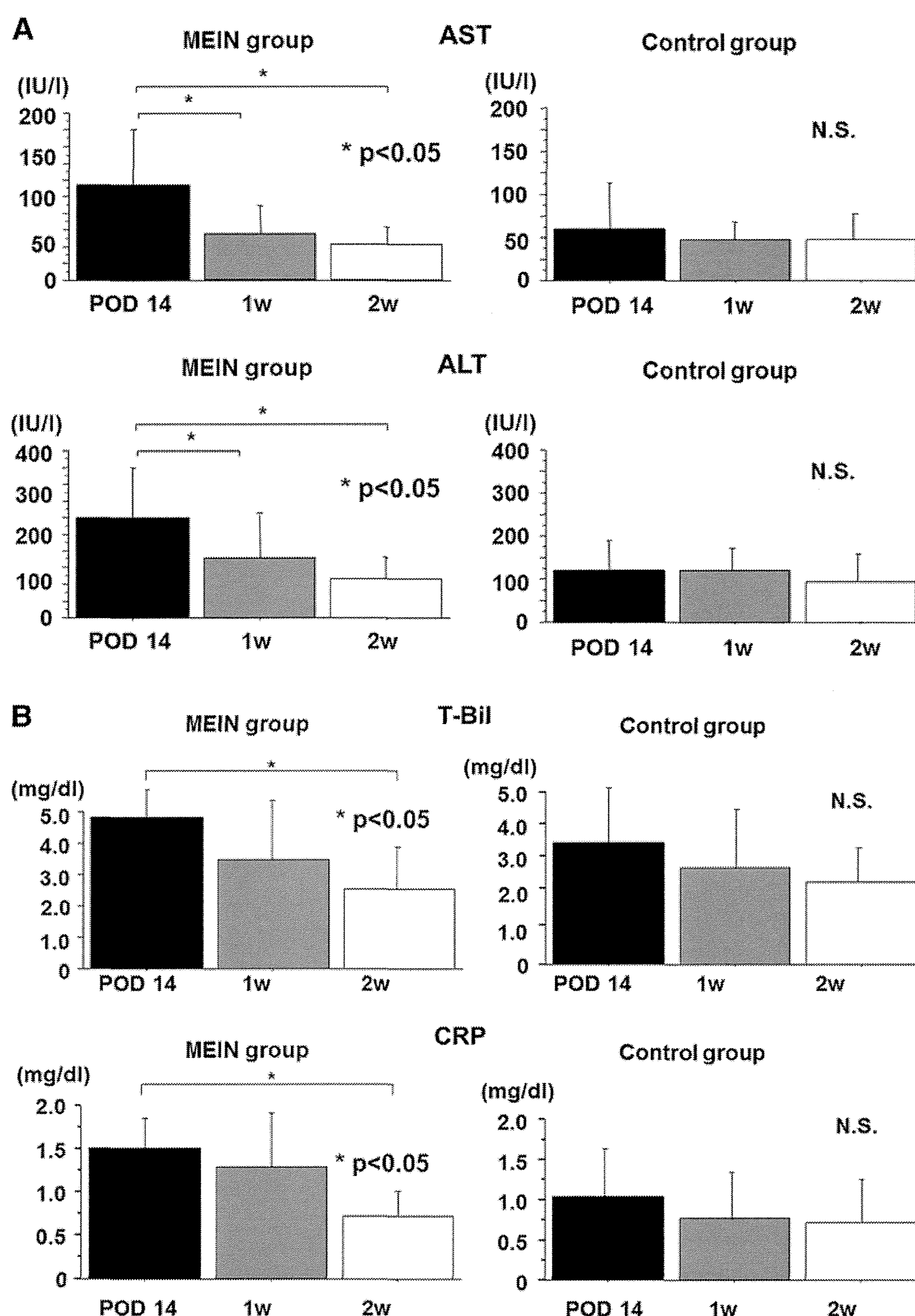

Fig. 2 The results of the biochemical analyses of the patients in the MEIN and control groups. **a** Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), **b** total bilirubin (T-Bil) and C-reactive protein (CRP) levels

Kume et al. [13] previously reported that whey-hydrolyzed protein has hepatoprotective effects against D-galactosamine-induced hepatitis and liver fibrosis in rats by suppressing IL-6. In the burn rat model, whey-hydrolyzed peptide led to a significant increase in hepatic glutathione levels 4 h after burn injury. The hepatic and renal lipid peroxide levels were increased 4 h after burn injury in the rats fed a standard diet. Whey supplementation significantly suppressed the burn-induced increase in the hepatic and renal lipid peroxide levels. Whey-hydrolyzed peptide also suppressed the hepatic and renal oxidative stress after experimental burn injury [14]. Recently, it was reported that MEIN® demonstrated anti-inflammatory effects and protected against concanavalin-A induced hepatitis in mice by suppressing the production of inflammatory cytokines [22].

The mucosal secretion of lactoferrin, which is composed of whey-hydrolyzed peptide, a glycoprotein present in milk, contributes to the host defense. Harversen et al. [15] have previously shown that orally given milk lactoferrin

mediates anti-infectious and anti-inflammatory activities in vivo. They also showed that lactoferrin could downregulate the lipopolysaccharide (LPS)-induced IL-6 secretion in a human monocytic cell line. Moreover, Hara et al. [12] reported that lactoferrin can also inhibit HCV and HBV infections in cultured human hepatocytes. Pre-incubation of the cells with bovine or human lactoferrin prevented the HBV infection of the cells. This report suggested that the interaction of lactoferrin with cells was important for its inhibitory effect, and that lactoferrin may be a candidate anti-HBV agent that could prove to be effective for the treatment of patients with chronic viral hepatitis.

In a recent clinical prospective study involving thirty adult patients, MEIN® was administered to ten patients who underwent LDLT and twenty patients (as controls) received a conventional enteral diet as the formula for early enteral nutrition. The incidence of bacteremia was significantly lower in the MEIN group than the control group (10 vs. 50 %, $p = 0.032$). The mean length of postoperative hospital stay after LDLT was significantly shorter in the MEIN group than that in the control group (45 ± 12 vs. 71 ± 34, $p = 0.018$) [23]. In a more recent study, it was shown that early administration of MEIN® could prevent post-transplant bacteremia in 76 consecutive patients [24].

Based on these previous studies and our current findings, we propose a flow chart for the management of patients with re-elevation of serum liver enzymes after LDLT, as shown in Fig. 3. If the patient shows re-elevation, diagnostic imaging, including US, CT or MRCP and blood tests should be performed to exclude blood flow disturbances, such as thrombosis or stenosis, bile duct complications or a recurrence of hepatitis virus infection. If the cause of the re-elevation is determined to be one of these etiologies, adequate management for such an etiology should be

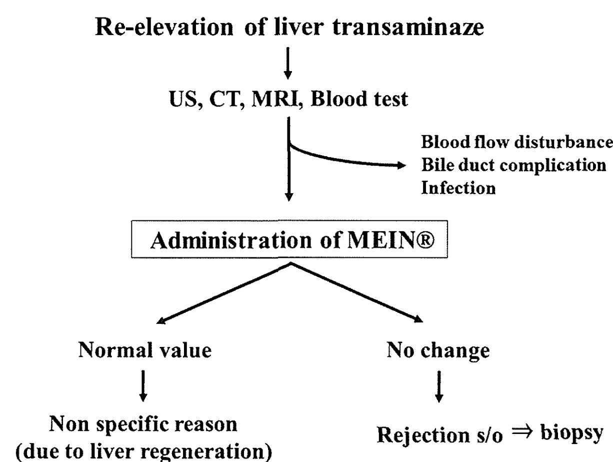

Fig. 3 A proposed flow chart of the postoperative management of patients who show a re-elevation of AST and ALT after LDLT

conducted. On the other hand, if the cause of the re-elevation cannot be clearly identified, then MEIN® should be administered. If the levels do not recover, a liver biopsy may be performed to rule out other etiologies, such as acute cellular rejection. However, since the number of patients included in this retrospective study was small, this flow chart should be confirmed in a prospective study involving a larger number of LDLT patients.

In conclusion, the administration of MEIN® can attenuate the re-elevation of liver enzyme levels after LDLT, and may help avoid the need for a liver biopsy.

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Effects of Pegylated Interferon α2b on Metastasis of Hepatocellular Carcinoma[1]

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Objective. **Interferon (IFN) has an anti-tumor activity in hepatocellular carcinoma (HCC) *via* anti-angiogenesis and induction of apoptosis. We have previously reported anti-metastatic effects of IFN combined chemotherapy on the outcome of HCC patients. The aim of this study was to investigate anti-metastatic effects of IFN.**

Methods. **In vitro, pegylated interferon α2b (PEG-IFN-α2b) was administered to mouse MH134 cells (mouse HCC cell line, MH134), and anti-implantation effects were examined by evaluating the inhibition of cell invasion and cell proliferation. Expressions of vascular endothelial growth factor (VEGF) mRNA were also measured. *In vivo*, PEG-IFN-α2b was subcutaneously administered into MH134 cells and tumor growth was evaluated. In distant metastasis models, PEG-IFN-α2b was subcutaneously administered and MH134 cells were injected into the spleen. The number of liver metastases and microvessel densities (MVD) were counted.**

Results. ***In vitro*, the proliferation of MH134 cells was significantly suppressed by PEG-IFN-α2b dose-dependently. MH134 cells added with PEG-IFN-α2b exhibited significantly lower levels of invasion potential. *In vivo*, tumor size in mice treated with PEG-IFN-α2b significantly suppressed compared with control mice (mean 0.5 *versus* 5.0 cm, in diameter, $P < 0.05$) and also decreased number of liver metastases (19.3 *versus* 6.0, $P < 0.05$). Moreover, PEG-IFN-α2b significantly suppressed angiogenesis compared with the control.**

Conclusion. **PEG-IFN-α2b in itself had remarkable anti-metastatic effects *via* inhibition of angiogenesis and cell adhesions.** © 2012 Elsevier Inc. All rights reserved.

Key Words: **hepatocellular carcinoma; metastasis; interferon.**

INTRODUCTION

Hepatocellular carcinoma (HCC), which is the main type of primary liver cancer, is one of the most common and aggressive malignancies. Hepatic resection is the standard treatment for HCC; however, the survival rate is still low because of the high incidence of recurrence [1]. Especially the tumor portal vein thrombus is poor prognostic factor due to intra- and extra-hepatic metastasis [2]. HCC is not generally sensitive to chemotherapy. Recently, new chemotherapy has appeared and interferon (IFN) has an anti-tumor activity in HCC *via* anti-angiogenesis and induction apoptosis for primary liver lesion [3–5]. We have already reported anti-metastatic effects of IFN combined chemotherapy (CDDP+5-FU) on the outcome of HCC patients [6]. There are some reports demonstrating the inhibitory effects on HCC after IFN therapy alone [7, 8]. However, mechanisms of anti-metastatic effects of IFN for HCC are still unclear. Cancer metastasis is the hallmark of malignant tumors. The progressive metastasis of malignant tumors depends mainly on angiogenesis and cell adhesion [9–11]. The aim of this study is to investigate the effects of IFN inhibiting HCC proliferation and metastasis *via* angiogenesis and cell adhesions.

MATERIALS AND METHODS

Cell Line and Animals

MH134, a mouse hepatocellular carcinoma cell line, was moderately differentiated and grows in synergenic recipients in both solid and ascitic forms [12]. The cell lines were kindly provided from Cell

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Resource Center for Biomedical Research, Institute of Development, Aging, and Cancer, Tohoku University (Sendai, Japan).

Six-wk-old male C3H/HEN Crj mice were obtained from Charles River Laboratories (Kanagawa, Japan). Animals were provided with water and standard laboratory diet for at least 7 d before use. Throughout the experiment, the animals were maintained behind barriers under controlled conditions and had free access to tap water and food. The present study was conducted in compliance with the Division for Animal Research Resources, Institute of Health Biosciences, and the University of Tokushima. The experiments and procedures were approved by the Animal Care and Use Committee of the University of Tokushima.

Interferon

Pegylated IFN α2b (PEG-IFN-α2b) was kindly provided by Schering-Plough K.K. (Osaka, Japan). The specific activity of PEG-IFN-α2b was 6.4×10^7 IU/mg protein.

Experiment 1 (*In Vitro*) MTT Assay

PEG-IFN-α2b was added to MH134 cells and anti-proliferative effects were examined by evaluating 3-(4, 5-dimethyithiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Chemicon International Inc., Temecula, CA, USA). The cells were seeded on 96-well plates (Coster; Corning Inc., Tokyo, Japan), cultured for 24 h, and the culture medium was changed to a new medium with or without PEG-IFN-α2b (1×10^3, 1×10^4, 1×10^5 IU/mL). After culturing for 24, 48, 72, or 96 h, the number of viable cells was measured with absorbance meter (F-2500; Hitachi, Tokyo, Japan) by setting the wavelength at 570 nm [13].

Cell Invasion Assay

PEG-IFN-α2b was added to MH134 cells and anti-invasion effects were examined by evaluating cell invasion assay (Cultrex 96 Well BME Cell Invasion Assay). The assay kit was adapted to multiple formats so that cell invasion might be evaluated against different extracellular matrices; laminin 1, collagen 1, and collagen 4. The cells were seeded on 96-well plates, cultured for 24 h, and the culture medium was changed to a new medium with or without PEG-IFN-α2b (1×10^3, 1×10^4, 1×10^5 IU/mL). After culturing for 24 h, the number of viable cells was measured with absorbance meter (F-2500; Hitachi) by setting the wavelength at 520 nm [14].

Quantitative Real Time RT-PCR for VEGF and bFGF in HCC Cells

The mRNA expression levels of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) were evaluated by quantitative real time reverse transcription polymerase chain reaction (RT-PCR). Total RNA was extracted from cultured MH134 cells in medium containing (1×10^3, 1×10^4, 1×10^5 IU/mL) PEG-IFN-α2b using RNeasy Mini Kit (Qiagen, Hilden, Germany). Quantitative real time RT-PCR was performed by using an ABI 7500 real-time PCR system (PE Applied Biosystems, Carlsbad, CA, USA). TaqMan gene expression systems (PE Applied Biosystems) for VEGF (assay ID Mm00437304) and bFGF (assay ID Mm01285715) were used for quantification of mRNA expression of the respective genes. To normalize, amplification of GAPDH (TaqMan ribosomal RNA control reagents, assay ID Rn9999916) was performed as an endogenous control.

Experiment 2 (*In Vivo*, Subcutaneous Injection Model)

The mice were divided into two groups; control group ($n = 10$) and IFN group ($n = 10$). Twenty-four hours before subcutaneous implantation of the MH134 cells (1×16^5 cells/mouse), IFN (1×10^3, 1×10^4, or 1×10^5 IU/body) was subcutaneously administered in each mouse. Tumor maximum diameter was measured in single direction using calipers 7 d after cell transplantation.

Experiment 3 (*in vivo*, Intrahepatic *Via* Portal Vein Metastasis Model) Intrahepatic and Portal Vein Metastasis Model (Splenic Injection Model)

Six-wk-old male C3H/HEN Crj mice were obtained from Charles River Laboratories, and were anesthetized with ether. A small upper-quadrant incision was made to expose the spleen. Using a 27-gauge needle, 1×10^5 cells/mouse was injected into the lower splenic pole. One week after splenic injection, the number of macroscopic metastases on the surface of the liver was counted [15]. Twenty-four hours before the splenic injection, IFN (1×10^3,1×10^4, or 1×10^5 IU/body) was subcutaneously administered in each mouse.

Quantification of Microvessel Density

Excised liver specimens were fixed in 10% formaldehyde and embedded in paraffin. Histologic study was performed on 4 μm sections stained with hematoxylin and eosin. Immunohistochemical staining was performed on 4 μm sections using the anti-mouse CD34 antibody (H-140; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Overnight incubation at 4°C with primary antibody and indirect immunoperoxidase staining with the avidin-biotin complex (DAKO, Glostrup, Denmark) and DAB Tablet (Wako Pure Chemical Industries, Ltd. Osaka, Japan) were applied for visualization of the antigens. Quantification of blood vessels was carried out as described previously [16]. Any brown-staining endothelial cell cluster distinct from adjacent microvessels, tumor cells, or other stromal cells was considered a single countable microvessel. Sections were examined at a magnification of ×400, and five fields were randomly chosen to determine the expression of CD34 by two pathologists who had no direct relation to this study. Expression of these proteins was evaluated as the number of positive staining cells.

Histology and Immunohistochemistry (ICAM and VCAM) in the HCC

Vascular cell adhesion molecule-1 (VCAM) and intercellular adhesion molecule-1 (ICAM) are expressed in the normal mouse hepatic sinusoid [17]. Excised liver specimens were fixed in 10% formaldehyde and embedded in paraffin. Histologic study was performed on 4 μm sections stained with hematoxylin and eosin. Immunohistochemical staining was performed on 4 μm sections using the anti-mouse ICAM antibody (10020-1-AP; ProteinTech Group, Inc., Chicago, IL) and anti-mouse VCAM antibody (sc-8304; Santa Cruz Biotechnology, Inc.). Overnight incubation at 4°C with primary antibody and indirect immunoperoxidase staining with the avidin-biotin complex (DAKO) and DAB Tablet (Wako Pure Chemical Industries, Ltd.) were applied for visualization of the antigens.

Statistical Analysis

All results were presented as mean ± standard deviation (SD). Student's t-test and Mann-Whitney U test were used for statistical analysis. P value < 0.05 was considered statistically significant.

RESULTS

MTT Assay

To evaluate the effect of PEG-IFN-α2b for MH134 cells, we performed MTT assay. Twenty-four hours after the

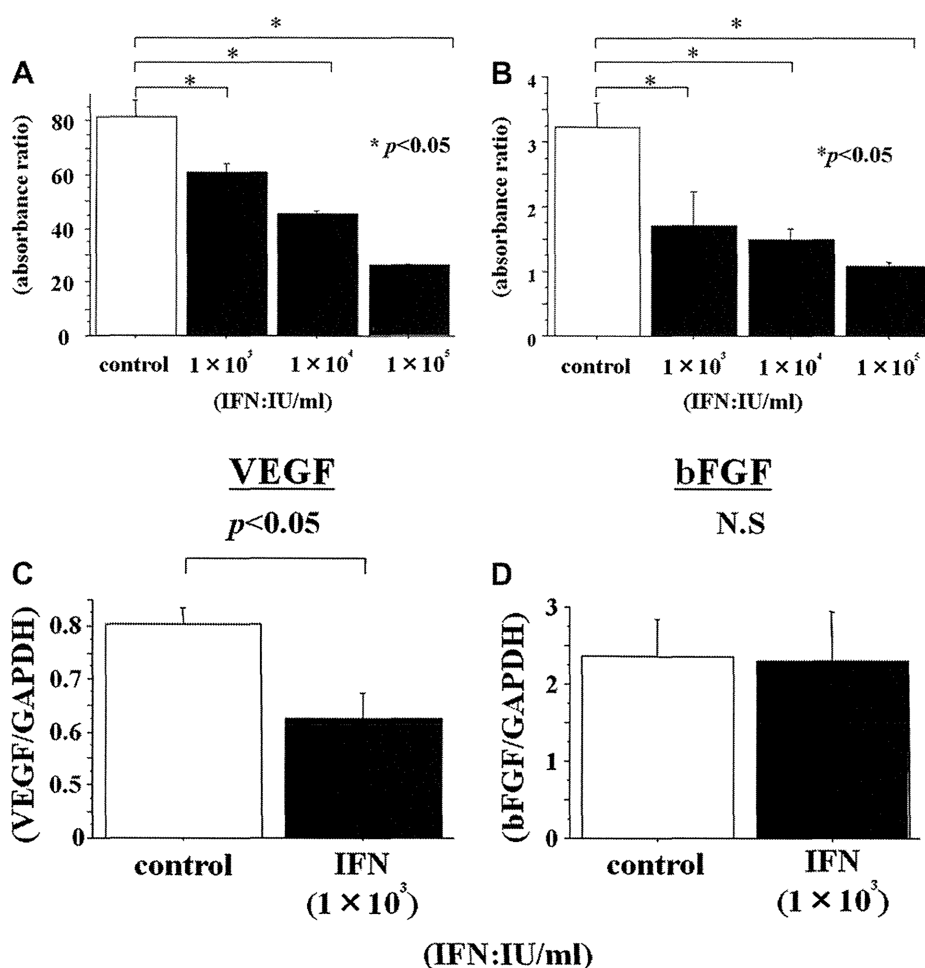

FIG. 1. (A) MTT assay showed antiproliferative effect of PEG-IFN-α2b. The proliferation of MH134 cells was suppressed dose-dependently ($P < 0.05$). (B) Invasion assay showed anti-invasive effect of PEG-IFN-α2b. The invasion of the matrigel-coated filters was suppressed dose-dependently ($P < 0.05$). (C) Expression of VEGF mRNA by quantitative real-time RT-PCR. (D) Expression of bFGF mRNA by quantitative real-time RT-PCR. *In vitro* inhibition of VEGF mRNA but not bFGF mRNA by PEG-IFN-α2b. mRNA was extracted in MH134 cells and those cells incubated in medium containing PEG-IFN-α2b ($P < 0.05$).

addition of PEG-IFN-α2b (1×10^3, 1×10^4, 1×10^5 IU/mL), the proliferation of MH134 cells was significantly suppressed by PEG-IFN-α2b dose-dependently compared with control group ($P < 0.05$, Fig. 1A).

Cell Invasion Assay

MH134 cells were examined in the invasion assay to determine if their ability to penetrate the reconstituted basement membrane matrigel. MH134 cells added with PEG-IFN-α2b (1×10^3, 1×10^4, 1×10^5 IU/mL) exhibited significantly lower levels of invasion potential (control *versus* PEG-IFN-α2b 1×10^3, 1×10^4, 1×10^5 IU/mL, mean 3.239 *versus* 1.707, 1.499, 1.047, Fig. 1B).

Quantitative Real Time-PCR for VEGF and bFGF in HCC Cells

Anti-angiogenic effects of PEG-IFN-α2b on MH134 cells by blocking the expression of VEGF and bFGF were studied. The expression of VEGF mRNA decreased in cells with PEG-IFN-α2b significantly ($P < 0.05$, Fig. 1C). The treatment with PEG-IFN-α2b resulted in a reduction of VEGF mRNA for 24 h. However, the expression of bFGF mRNA in the cells with PEG-IFN-α2b did not differ significantly compated with the control ($P > 0.05$, Fig. 1D).

Tumor Diameter

Dose-dependent suppression of tumor diameter was observed in mice receiving PEG-IFN-α2b. PEG-IFN-α2b significantly suppressed compared with control mice ($P < 0.05$, Fig. 2).

Liver Metastasis

Macroscopic metastasis of all mice could be seen on the surface of the liver. PEG-IFN-α2b significantly

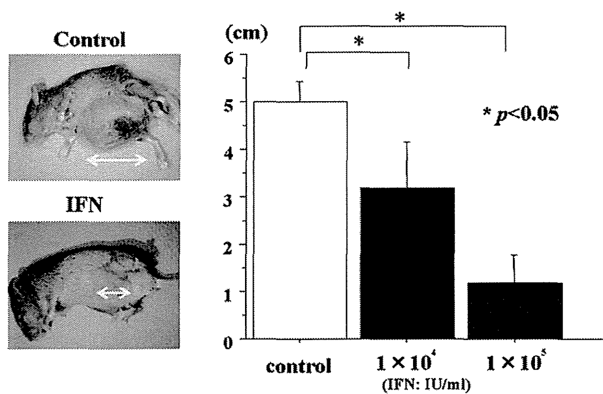

FIG. 2. Dose-dependent change in tumor diameter of subcutaneously injection model. The mice received a subcutaneous injection of 1×10^4 and 1×10^5 IU/mL PEG-IFN-α2b. PEG-IFN-α2b significantly suppressed tumor growth ($P < 0.05$). (Color version of figure is available online.)

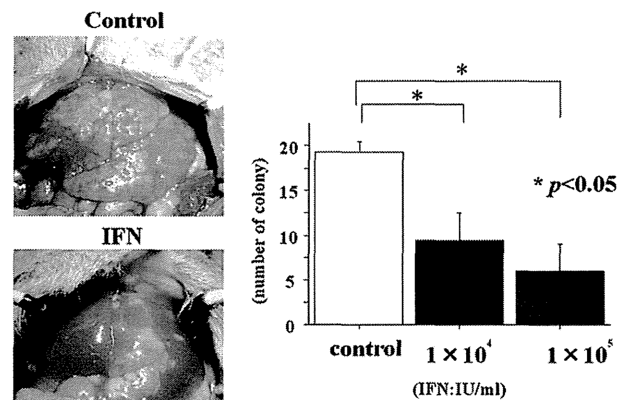

FIG. 3. Intrahepatic metastasis *via* portal vein model showed number of liver metastasis nodule seven days after operation. The mice received a subcutaneous injection of 1×10^4 and 1×10^5 IU/mL PEG-IFN-α2b. PEG-IFN-α2b significantly decreased number of liver metastasis ($P < 0.05$). (Color version of figure is available online.)

decreased the number of liver metastases (19.3 *versus* 6.0, $P < 0.05$, Fig. 3).

Tumor Angiogenesis (Microvessel Density)

The number of blood vessels was quantified by counting the stained regions in five high-power fields (×400). PEG-IFN-α2b significantly suppressed angiogenesis compared with control mice ($P < 0.05$, Fig. 4).

Expressions of VCAM and ICAM Protein

These pictures show non-tumorous area of mouse liver. Immunohistochemically, they showed weak positive reaction in the entire field, so both the VCAM and ICAM expressions showed no difference between the metastatic tumors or the non-tumorous liver tissues (Fig. 5).

DISCUSSION

The metastasis of cancer cells has a multi-step and key-molecule so-called 'seed and soil' theory [18]. The steps of metastasis are characterized by cells that lose their cell–cell contact (E-cadherin, β-catenin), cross basement membrane [matrixmetaloprotease (MMP) family], invade stroma (MMP family), spread across blood vessels, adhere vascular endothelial cells (Sialyl-Lewis X, integrin, ICAM, VCAM) and form new neoplastic tissue and angiogenesis (integrin, VEGF, bFGF, angiopoietin 2) in sites other than that of the original tumor.

IFN has been shown to reduce the incidence of preneoplastic foci and cancer in HCC model [19, 20]. IFN has been already reported to inhibit the growth of a variety of cancer cells, including multiple myeloma, ovarian cancer, and liver cancer cells [21]. In HCC, IFNα is reported to up-regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in T cells, NK cells and monocytes [3], and Fas/Fas ligand pathway [5]. It also exerts immunomodulatory effects by stimulating T cells, NK cells, and monocytes [3].

In this study, we use the mouse cell lines. The effect of human IFNs on murine tumor cells *in vivo* has already been reported [22]. First, we demonstrate that PEG-IFN-α2b can inhibit the invasion of floating MH134 HCC cells. Second, we can demonstrate the anti-metastatic effect of PEG-IFN-α2b with *in vivo* intrahepatic and portal vein metastasis model showing the reduction of the liver metastases. So these results can cause the speculation about the anti-metastatic effect of PEG-IFN-α2b. However, few studies have reported the anti-metastatic effects of IFNα, and the mechanisms of these effects are still unclear. The invasiveness

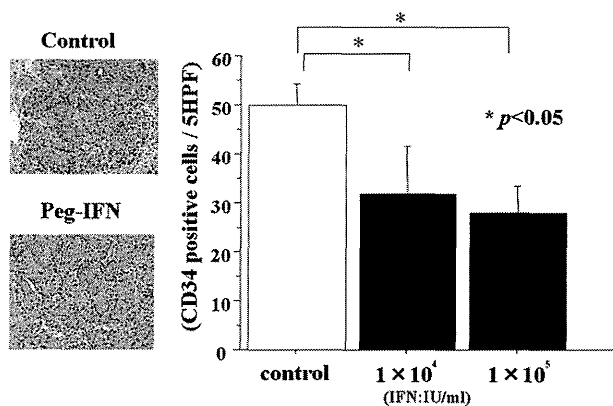

FIG. 4. Microvessel density in metastatic liver tumor. Detection of CD34 positive cells by immunohistochemical staining (×400). Any brown-staining cell cluster distinct from adjacent microvessels, tumor cells, or other stromal cells was considered a single countable microvessel, and the number of CD34 positive cells was significantly lower compared with the control group ($P < 0.05$). (Color version of figure is available online.)

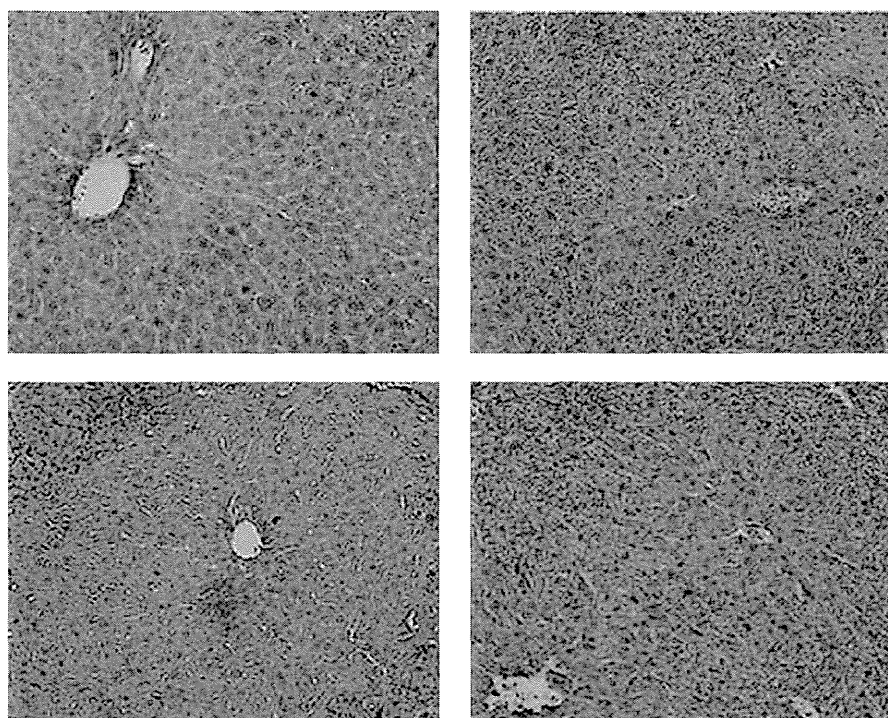

FIG. 5. Detection of VCAM and ICAM proteins in liver tissue by immunohistochemical staining (×400). There was no difference compared with the control group. Positive expression of VCAM and ICAM was almost not found at liver. (Color version of figure is available online.)

of tumor cells represents one of several important properties that are necessary for the formation of metastasis [23]. The cell invasion kit is created in an effort to accelerate the screening process for compounds that influence cell migration through extracellular matrices, which is a fundamental function of cellular processes such as angiogenesis, embryonic development, immune response, and metastasis of cancer cells [14].

On the other hand, angiogenesis is essential for cancers to metastasize. We have demonstrated that PEG-IFN-α2b inhibits the mRNA expression levels of VEGF in mouse HCC cells. These MH134 cells have been shown to produce endogenous VEGF [24]. IFN has been shown to down-regulate the expressions of the major stimulatory molecules, such as bFGF, VEGF, interlukin-8, MMP-2, and MMP-9, and to inhibit angiogenesis in most malignant tumors [25]. Continuous contact with exposure to 1000 IU/mL PEG-IFN-α2b induces strong antitumor effects in HCC cells [13]. The concentrations of PEG-IFN-α2b used in this study are almost identical to those of another study showing the inhibitory effects of 1000–3000 IU/mL IFNα on the VEGF mRNA expression in the human HCC cell line [25]. Tumor stromal cells might also provide angiogenic signaling, such as MMPs, which interact with tumor cells to stimulate angiogenesis [26]. In response to angiogenic signaling, tumor cells secrete a group of pro-angiogenetic polypeptides, known as angiogenic factors, which trigger the formation of neovasculature from the host vessels. VEGF is one of the first isolated angiogenic peptides and the most studied angiogenic factor so far. It has a specific mitogenic effect on endothelial cells, and it also increases vascular permeability and promotes extravasation of proteins from tumor vessels, leading to the formation of a fibrin matrix that supports the growth of endothelial cells and allows invasion stromal cells into the developing tumor [27]. The effects of VEGF are mediated *via* its receptors, VEGF-1 (Flt-1) and VEGF-2 (KDR), in endothelial cells [28]. VEGF appears to play a significant role in the early stage of hepatocarcinogenesis. Its expression increases gradually from low-grade dysplastic nodules to high-grade dysplastic nodules to early HCC [29]. FGF is a family of heparin-binding growth factor that includes at least 22 structurally related members, of which bFGF is the best known member. Tumor cell-derived bFGF that acts as a paracrine endothelial mitogen, and endothelial cells themselves, produce and release bFGF, which acts in an autocrine fashion

independently. The bFGF appears to act synergistically with VEGF in the induction of angiogenesis [30, 31]. However, in the current study, the mRNA expression of bFGF dose not change in several different dosages (data not shown). In fact, there is no report demonstrating that IFN inhibits bFGF expression.

The intratumor micro vessel density (MVD) in the IFN group is lower than in the control group, possibly resulting from the inhibition of the VEGF expression by IFN. The intratumor MVD is a direct reflection of tumor angiogenesis. It can be visualized by immunohistochemical staining with antibodies to anti-CD34 and α-smooth muscle actin [32]. MVD levels have a close relationship with intrahepatic recurrence, disease-free survival, and could be a predictive marker for disease-free survival [33].

Cell adhesion is also an important process of metastasis, and several adhesion factors, E-cadherin, vascular cell adhesion molecule-1 (VCAM), intercellular adhesion molecule-1 (ICAM), integrin family are related with cell adhesion. The expression level of E-cadherin invasively correlates with HCC histologic grade and prognosis. E-cadherin underexpression might have some contribution to the early recurrence of HCC [34]. It has been reported that focal adhesion kinase and E-cadherin appear to be significantly up-regulated after exposure to retinoic acid with IFN on SCC [35]. However, it is not shown in the primary tumor but in metastatic tumor in this study. ICAM and VCAM are important for tumor cells first adhesion to the endothelium as 'soil' factor and, therefore, recurrence or metastasis [36]. Serum concentration of intercellular adhesion molecule-1 (sICAM-1) in patients with HCC is a marker for disease progression and prognosis. Higher sICAM levels are more frequently observed in the patients with multiple lesions and intrahepatic metastasis, and have a poor prognosis. Detecting sICAM-1 is of important value in predicting tumor recurrence after surgery [37]. However, ICAM and VCAM are not significantly expressed in the liver as 'soil' factor in this study. Additional experiments might be required regarding other cell adhesion molecules, such as MMP, Sialyl-Lewis X, and integrin.

In conclusion, IFN in itself has remarkable anti-metastatic effects. These findings suggest a mechanism by which IFN inhibits angiogenesis and cell invasion in HCC.

ACKNOWLEDGMENTS

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Case Report

Successful Case with Hemophagocytic Syndrome after Living Donor Liver Transplantation

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SUMMARY

Hemophagocytic syndrome (HPS) is a rare but serious complication that is associated with hypercytokinemia caused by activated T lymphocytes and macrophages in immunologically compromised patients. Living donor liver transplantation (LDLT) between adults has been performed to compensate for the shortage of available organs. There have been some reports of HPS after LDLT but its prognosis is disappointingly poor. Herein, we report a case of HPS in a 53-year-old woman who underwent LDLT using a left lobe graft. HPS was diagnosed on postoperative day 6 and successfully treated with a steroid pulse. HPS is a fatal complication in immunologically compromised patients but its early diagnosis and appropriate treatment can lead to an improved outcome.

Key Words: Hemophagocytic syndrome; Living donor liver transplantation.

INTRODUCTION

Hemophagocytic syndrome (HPS) is a rare but serious hematological disorder in immunocompromised patients. The disorder presents with a variety of symptoms, the most common of which are fever lymphadenopathy, hepatosplenomegaly, jaundice and skin rash. HPS patients frequently exhibit abnormal laboratory values including pancytopenia, liver dysfunction, coagulopathy, hypofibrinogenemia, hypertriglyceridemia and hypocholesterolemia. The background of this condition is hypercytokinemia, which is mainly caused by infectious agents such as cytomegalovirus (CMV), Epstein-Barr virus (EBV) and bacteria (1). Histological findings of phagocytosis of hematopoietic precursor cells in the bone marrow, lymph nodes, liver or spleen provide a conclusive diagnosis. The prognosis of HPS has been reported to be poor. Although HPS is a rare complication after liver transplantation, its prognosis when it occurs was reported to be 50% (1-6). Only a small number of patients with HPS have been reported after liver transplantation, despite the fact that most patients with HPS are immunocompromised.

Living donor liver transplantation (LDLT) between adults has been performed to compensate for the shortage of available organs. There have been some reports of HPS after LDLT, but its prognosis was disappointingly poor (1,7). Herein, we report a patient who developed HPS following LDLT and was successfully treated with a steroid pulse.

CASE REPORT

A 53-year-old woman underwent LDLT for hepatocellular carcinoma with decompensated liver cirrhosis owing to hepatitis C viral infection. The graft was a left lobe graft (graft weight, 510g; GV/SLV, 49%) which was donated by her 21-year-old son. The procedure itself was uneventful, with an estimated blood loss of 3090g. Immunosuppressive drugs consisted of induction with basiliximab on days 0 and 4 and mycophenolate mofetil and methylprednisolone followed by cyclosporine starting at 7 days after the transplant. The immediate graft function was excellent and the patient was extubated on the following day. However, a high fever accompanied by pancytopenia developed on postoperative day 6 and persisted thereafter, despite the administration of antibiotics. Other than the high fever, the patient's general condition was unexpectedly stable with excellent graft function. Infectious work-ups including systemic CT scans, bacteriological and fungal cultures and other laboratory examinations were negative for infectious disease. On the basis of these findings, a bone marrow tap was performed and HPS was finally diagnosed (**Figure 1**). The serum ferritin level was significantly elevated (304ng/mL). A steroid pulse with taper was im-

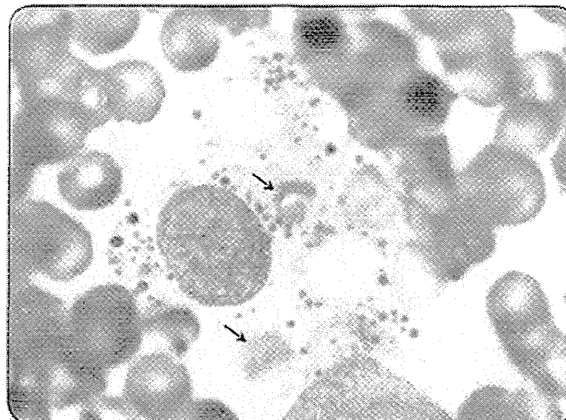

FIGURE 1. Hemophagocytosis in the bone marrow. A bone marrow smear taken on postoperative day 6 shows prominent activated macrophages which have ingested red blood cells and neutrophilic leukocytes. Arrows: neutrophilic leukocytes.

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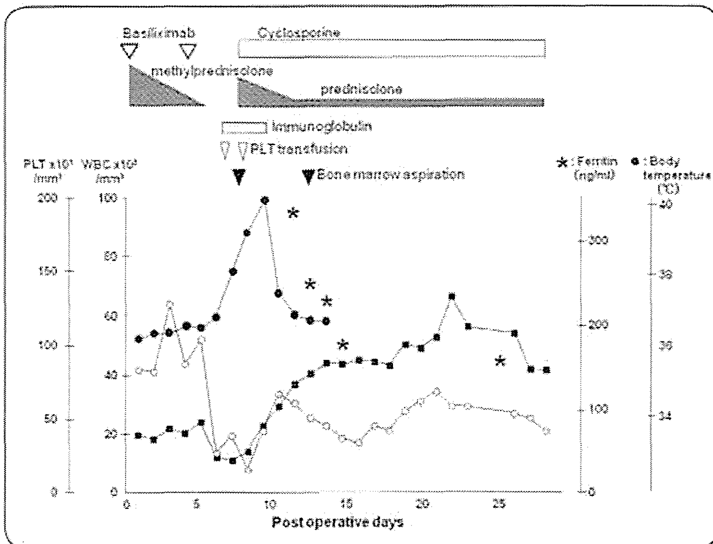

FIGURE 2. Postoperative course of blood cell count and details of the treatment. WBC: White Blood Cells; PLT: Platelets.

mediately started, which resulted in rapid alleviation of the fever and pancytopenia. The postoperative course is shown in **Figure 2**. The patient recovered rapidly without any other complications and returned home at 50 days after the LDLT.

DISCUSSION

HPS is a rare but severe hematological complication that occasionally develops in patients in various situations, including complicated liver transplantation. Common examples include lymphoma-associated hemophagocytic syndrome, virus-associated hemophagocytic syndrome (VAHS), bacteria-associated hemophagocytic syndrome and autoimmune-associated hemophagocytic syndrome. The most frequent type is VAHS associated with CMV, EBV, adenovirus, Coxsackie virus B5, parvovirus B19 or human herpes virus 6 (1). Although viral infection is a frequent complication in transplant recipients, very few cases of HPS after liver transplantation have been reported, suggesting a low level of awareness of this hematological disorder.

In our patient, CMV antigenemia was negative in both the preoperative and postoperative periods. EBV infection was also negative in preoperative screening. A severe infection that caused the hypercytokinemia and activation of macrophages was not suspected from the findings, including systemic CT scans, bacteriological and fungal cultures and other laboratory examinations. Quantitative measurements of interferon-g, soluble interleukin-2 receptor, ferritin and beta-2-microglobulin are useful predictors of the prognosis but may not be practical for serial monitoring (7-12). In the present case, the serum ferritin level was significantly elevated. Consequently, HPS was suspected and we planned the use of a steroid. However, an infectious disorder had not been ruled out completely and a bone marrow tap was performed before the steroid treatment (13). Bone marrow cytology revealed that the cause of the pancytopenia and high fever was HPS and we therefore began treatment with steroid and cyclosporine administration. The patient recovered rapidly after the treatment without any other complications.

Although the pathogenesis of HPS is not fully understood, the condition may be initiated by activated T cell-mediated immune responses to various primary conditions including familial disorders, infections, neoplasms and autoimmune diseases. The absence of a family history of HPS in our patient (including the donor) ruled out the familial form of PHS. Among the underlying causes, viral infection is the most frequent and is referred to as VAHS. Although the definite cause of the HPS remained obscure in our patient, a persistent infectious state before transplantation could have caused hypercytokinemia, which may have triggered the HPS in the early postoperative period (on postoperative day 6).

In patients with HPS, uncontrolled T cell proliferation is followed by overproduction of cytokines, including interferon-gamma, interleukin-6, tumor necrosis factor-alpha, macrophage colony-stimulating factor and soluble interleukin-2 receptor, by the unregulated T lymphocytes. These cytokines account for the activation of macrophages that are subsequently transformed to hemophagocytes and are also responsible for the variety of clinical manifestations and laboratory data in HPS. Baseline immunosuppression may have affected this cytokine profile. Although it should be emphasized that awareness and early diagnosis of HPS are required to institute timely treatments to reduce its fatality, clinical data are not necessarily helpful in establishing the early diagnosis because the features are usually non-specific. Although a prompt bone marrow biopsy is recommended for the diagnosis of HPS, measurements of serum cytokine levels as relatively specific markers seem to contribute most to the prediction of disease progression or regression.

Recently, several authors have focused on the relevance of elevated levels of serum ferritin and urine and serum beta-2-microglobulin, which are known reactive proteins derived from activated macrophages, natural killer cells and T cells, in patients with HPS. Although the values for beta-2-microglobulin should be interpreted with caution because it may be produced during the process of alloimmunity or excessively filtered into the urine owing to immunosuppressant-induced nephrotoxicity in post-transplantation patients, these levels can be regarded as surrogate markers of the serum cytokine levels.

However, it is imperative to establish the diagnosis with a bone marrow biopsy as early as possible to improve the outcome of this life-threatening condition. The indication criteria for a bone marrow biopsy include high fever, unexplained cytopenia, hypocholesterolemia, hypertriglyceridemia and hyperferritinemia. Recipients with unexplained high fever and pancytopenia should be suspected for the development of HPS. Early detection of HPS and prompt treatment are vital to overcome this complication.

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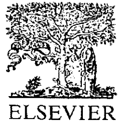

ELSEVIER

Preemptive Antiviral Treatment for Hepatitis C Virus After Living Donor Liver Transplantation

Y. Sugawara, S. Tamura, N. Yamashiki, J. Kaneko, T. Aoki, Y. Sakamoto, K. Hasegawa, and N. Kokudo

ABSTRACT

Background. Recurrence following liver transplantation for hepatitis C virus (HCV), which is universal, affects long-term outcomes. Treatment with interferon (IFN) and ribavirin (RBV), the only widely available options at this time, have been faced with low tolerability and overall unsatisfactory results in deceased donor liver transplantation (DDLT). However, its place after living donor liver transplantation (LDLT) remains a matter of debate. Since most LDLT cases are performed in a planned manner at a lower Model for End-stage Liver Disease (MELD) score compared to DDLT, we have aggressively applied preemptive INF/RBV in our series.

Patients and methods. We studied 122 adult recipients who underwent LDLT for HCV-related end-stage liver disease. The preemptive IFN/RBV protocol initiated treatment promptly after improvement in the patient's general condition with a low-dose IFN alpha2b and RBV (400 mg/d) followed by a gradual increase in the INFalpha2b dosage. Finally, we applied pegylated IFN (1.5 ug/kg/wk) and RBV (800 mg/d). The treatment was continued for 12 months after serum HCV-RNA became negative, which was defined as the end-of-treatment response (ETR). The response was considered to be a sustained viral response (SVR) if there were negative serologic results without antiviral treatment for another 6 months. Splenectomy was performed at the time of LDLT to improve tolerability to INF/RBV. The median age of the patients was 55 yrs (range = 23–66), with male dominance (87 males and 35 females). Median MELD score was 14 (range = 6–48). The series included 72 patients with hepatocellular carcinomas, and six with HIV coinfections. In 98 cases, HCV genotype was 1b.

Results. Overall survival at 5 years was 79%. Cumulative response rates under the protocol were ETR 56% and SVR 44% at 5 years.

Conclusions. Preemptive IFN/RBV therapy after LDLT for HCV is feasible with acceptable outcomes.

Hepatitis C virus (HCV) is one of the major causes of chronic liver disease, cirrhosis, and liver cancer in most developed countries,[1] including Japan.[2] Around one-third of recipients develop HCV-related cirrhosis in developed nations.[3, 4]

Liver transplantation is an effective treatment to reduce morbidity and mortality among this population. Reinfection with HCV, however, is a critical complication with major effects on graft and patient survivals. Controlling HCV begins after liver transplantation. In this article, we have

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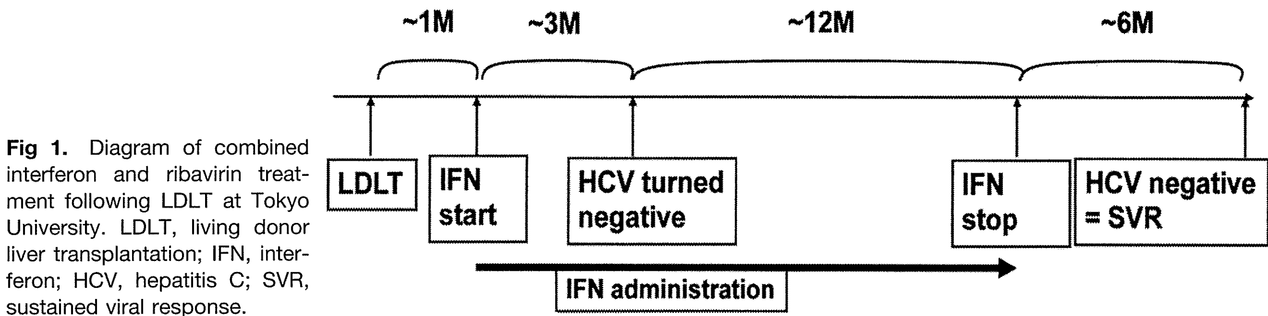

Fig 1. Diagram of combined interferon and ribavirin treatment following LDLT at Tokyo University. LDLT, living donor liver transplantation; IFN, interferon; HCV, hepatitis C; SVR, sustained viral response.

focused on preemptive strategies against HCV after transplantation to prevent reinfection with the goal of eradicating recurrent infections.

PATIENTS

We examined the feasibility and efficacy of a preemptive combination of interferon (IFN) and ribavirin (RBV) therapy against HCV in living donor liver transplantation (LDLT) patients.[5] Between January 1996 and July 2011, 122 subjects underwent LDLT for HCV. We studied prospectively the clinical courses of these patients whose median age was 55 years (range = 23–66) and the majority were males (87 men and 35 women) with an HCV genotype 1b (n = 98; 80%). The median Model for End-Stage Liver Disease score was 14 (range = 6–48). Six patients were coinfected with HIV and 72 (59%) showed hepatocellular carcinomas, 50 of which were within the Milan criteria.

Treatment was initiated with low-dose IFN alpha2b and RBV (400 mg/d) promptly after improvement in general condition following liver transplantation, especially recovery of hematologic and renal functions; leukocytes $\geq$ 4,000/mL; platelets $\geq$ 50,000/mL; hemoglobin $\geq$ 8 g/L, and serum creatinine $<$ 2 mg/dL. Thereafter, the dosage was gradually increased as tolerated. Finally, pegylated IFN (1.5 μg/kg/wk) and RBV (800 mg/d) were administered, depending on patient compliance, and continued for 12 months after the serum HCV-RNA became negative, which was defined as an end-of- treatment response (ETR). The response was considered to be a sustained viral response (SVR) if there were negative serologic results for another 6 months without antiviral treatment (Fig 1). Serologic monitoring for HCV-RNA was continuously performed on a monthly basis even after SVR was achieved to avoid unrecognized episodes or delayed diagnosis of relapse. Flexible dose adjustments were made to avoid serious adverse events and to prevent a lapse in treatment.

RESULTS

A total of 63 (52%) patients displayed negative HCV-RNA results at least once; of these, 42 patients (34%) experienced a sustained response for 12 months (ETR). Six patients who reached ETR eventually presented with a viral relapse and did not achieve an SVR. At the time of data collection, 32 (34%) achieved an SVR. None of the recipients who achieved an SVR have presented with a viral relapse. The median times to achieve a negative HCV-RNA, ETR, and SVR under the treatment regimen were 12 months (range = 2–63 months), 25 months (range = 13–79 months), and 28 months (range = 19–67 months), respectively.

At 5 years, negative HCV-RNA status was obtained in 65%, ETR in 56%, and SVR in 44% (Fig 2). HCV genotype 1b, use of cyclosporine, and a lower rate of tolerated RBV dose were associated with significantly poorer outcomes. Multivariate analysis revealed HCV genotype 1b as the only independent factor resulting in a significantly poorer (no SVR) viral response hazard ratio 0.339, 95% confidence interval 0.141 to 0.816 (P = .02).

The overall midterm rates of survival were not significantly different between HCV and non-HCV recipients. Analysis of factors affecting short-term survival rates indicated that viral titer prior to transplantation, viral response to treatment, acute cellular rejection episodes, donor age, and donor sex were significant factors affecting 2-year survival. Multivariate analysis revealed that a higher viral titer prior to transplantation, a poor response to antiviral treatment, occurrence of an acute cellular rejection episode, and older donor age were independent significant factors associated with poor survival (Fig 3).

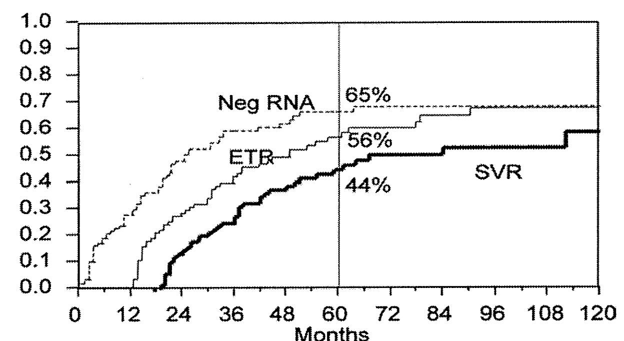

Fig 2. Cumulative viral response following preemptive treatment after living donor liver transplantation. SVR, sustained viral response; Neg RNA, Negative hepatitis C virus-RNA; ETR, end-of-treatment response.