(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).

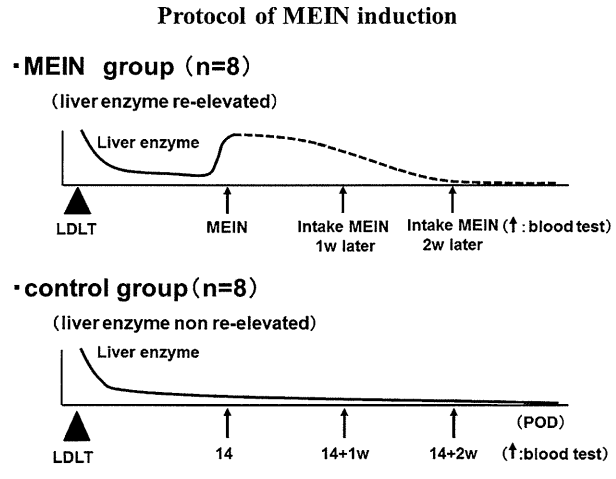

Fig. 1 The timing of the re-elevation of liver enzyme levels and the administration of MEIN

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.

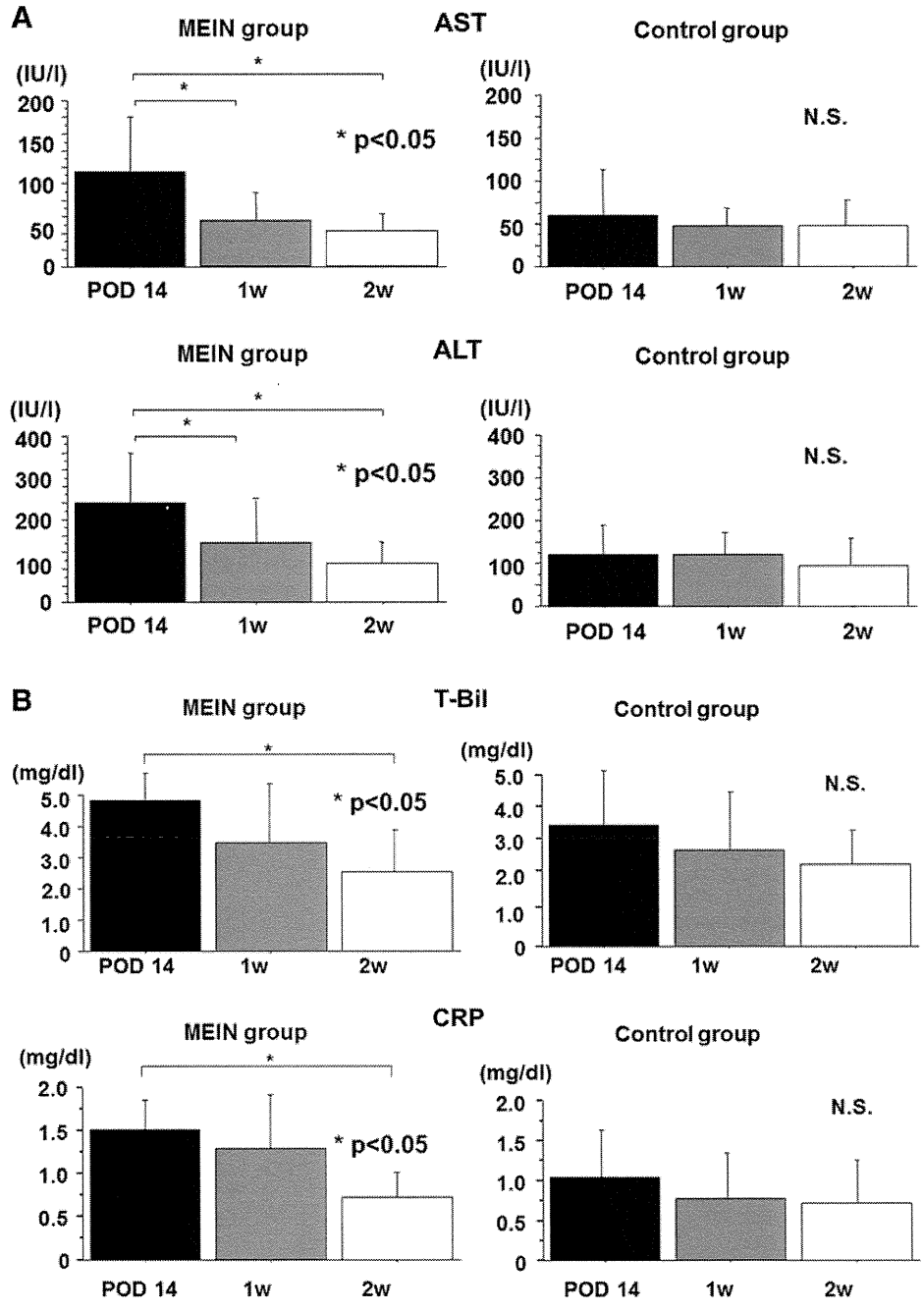

Fig. 2 The results of the biochemical analyses of the patients in the MEIN and control groups. **a** Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), **b** total bilirubin (T-Bil) and C-reactive protein (CRP) levels

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 down-regulate 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 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.

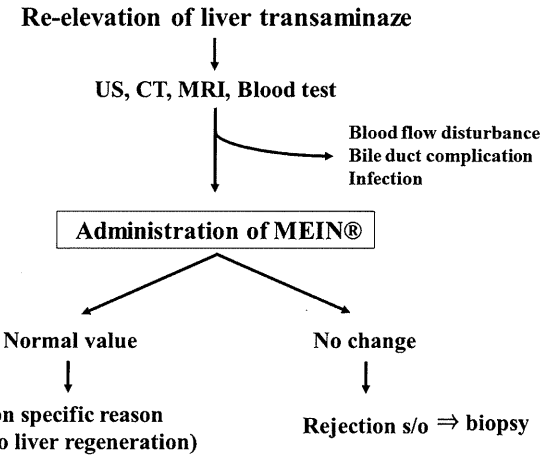

Fig. 3 A proposed flow chart of the postoperative management of patients who show a re-elevation of AST and ALT after LDLT

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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References

1. Taniai N, Onda M, Tajiri T, Akimaru K, Yoshida H, Yokomuro S, et al. Graft survival following three occurrences of hepatic arterial thrombosis after living-related liver transplantation. A case report. Hepato-gastroenterology. 2002;49:1420–2.
2. Greif F, Bronsther OL, Van Thiel DH, Casavilla A, Iwatsuki S, Tzakis A, et al. The incidence, timing, and management of biliary tract complications after orthotopic liver transplantation. Ann Surg. 1994;219:40–5.
3. Demetris AJ, Batts KP, Dhillon AP, et al. Banff schema for grading liver allograft rejection: an international consensus document. Hepatology. 1997;25:658–63.
4. Horoldt BS, Burattin M, Gunson BK, Bramhall SR, Nightingale P, Hubscher SG, et al. Does the Banff rejection activity index predict outcome in patients with early acute cellular rejection following liver transplantation? Liver Transpl. 2006;12:1144–51.
5. Wojcicki M, Milkiewicz P, Silva M. Biliary tract complications after liver transplantation: a review. Dig Surg. 2008;25:245–57.
6. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC, Smith AD. Liver biopsy. Hepatology. 2009;49:1017–44.
7. Prata Martins F, Bonilha DR, Correia LP, Paulo Ferrari A. Obstructive jaundice caused by hemobilia after liver biopsy. Endoscopy 2008;40(Suppl 2):E265–6.
8. Li F, Mekeel KL, Eleid M, Harrison ME, Reddy KS, Moss AA, et al. Hemobilia and pancreatitis after liver transplant biopsy. Liver Transpl. 2009;15:350–1.
9. Takeda K, Tanaka K, Kumamoto T, Nojiri K, Mori R, Taniguchi K, et al. Emergency versus elective living-donor liver transplantation: a comparison of a single center analysis. Surg Today. 2012;42:453–9.
10. Plank LD, McCall JL, Gane EJ, Rafique M, Gillanders LK, McIlroy K, et al. Pre- and postoperative immunonutrition in patients undergoing liver transplantation: a pilot study of safety and efficacy. Clin Nutr. 2005;24:288–96.
11. Marshall K. Therapeutic applications of whey protein. Altern Med Rev. 2004;9:136–56.
12. Hara K, Ikeda M, Saito S, Matsumoto S, Numata K, Kato N, et al. Lactoferrin inhibits hepatitis B virus infection in cultured human hepatocytes. Hepatol Res. 2002;24:228.
13. Kume H, Okazaki K, Sasaki H. Hepatoprotective effects of whey protein on D-galactosamine-induced hepatitis and liver fibrosis in rats. Biosci Biotechnol Biochem. 2006;70:1281–5.
14. Oner OZ, Ogunc AV, Cingi A, Uyar SB, Yalcin AS, Aktan AO. Whey feeding suppresses the measurement of oxidative stress in experimental burn injury. Surg Today. 2006;36:376–81.

15. Haversen L, Ohlsson BG, Hahn-Zoric M, Hanson LA, Mattsby-Baltzer I. Lactoferrin down-regulates the LPS-induced cytokine production in monocytic cells via NF-kappa B. Cell Immunol. 2002;220:83–95.
16. Watanabe A, Okada K, Shimizu Y, Wakabayashi H, Higuchi K, Niiya K, et al. Nutritional therapy of chronic hepatitis by whey protein (non-heated). J Med. 2000;31:283–302.
17. Lee YM, Skurk T, Hennig M, Hauner H. Effect of a milk drink supplemented with whey peptides on blood pressure in patients with mild hypertension. Eur J Nutr. 2007;46:21–7.
18. Ikeda M, Sugiyama K, Tanaka T, Tanaka K, Sekihara H, Shimotohno K, et al. Lactoferrin markedly inhibits hepatitis C virus infection in cultured human hepatocytes. Biochem Biophys Res Commun. 1998;245:549–53.
19. Bounous G, Batist G, Gold P. Whey proteins in cancer prevention. Cancer Lett. 1991;57:91–4.
20. Kaido T, Mori A, Ogura Y, Hata K, Yoshizawa A, Iida T, et al. Impact of enteral nutrition using a new immuno-modulating diet after liver transplantation. Hepatogastroenterology. 2010;57:1522–5.
21. Uchiyama H, Shimada M, Imura S, Morine Y, Kanemura H, Arakawa Y, et al. Living donor liver transplantation using a left hepatic graft from a donor with a history of gastric cancer operation. Transpl Int. 2010;23:234–5.
22. Kume H, Okazaki K, Yamaji T, Sasaki H. A newly designed enteral formula containing whey peptides and fermented milk product protects mice against concanavalin A-induced hepatitis by suppressing overproduction of inflammatory cytokines. Clin Nutr. 2012;31:283–9.
23. Kaido T, Mori A, Ogura Y, Hata K, Yoshizawa A, Lida T, et al. Impact of enteral nutrition using a new immuno-modulating diet after liver transplantation. Hepato-gastroenterology. 2010;57:1522–5.
24. Kaido T, Ogura Y, Ogawa K, Hata K, Yoshizawa A, Yagi S, et al. Effects of post-transplant enteral nutrition with an immunomodulating diet containing hydrolyzed whey peptide after liver transplantation. World J Surg. 2012.

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Article

Serial Changes of Serum Growth Factor Levels and Liver Regeneration after Partial Hepatectomy in Healthy Humans

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Abstract: This study aimed to investigate the associations of the serial changes of serum levels of various growth factors with liver regeneration after hepatectomy in healthy liver donors. Sixteen healthy liver donors who underwent conventional liver resection were included. Serum levels of various growth factors before hepatectomy and on postoperative day (POD) 1, 3, 5 and 7 were measured. Liver volume data calculated by multi-detector computed tomography using workstation. The ratio of remnant liver volume on POD 0 to liver volume before the operation was 51% ± 20%. The ratio of liver volume on POD 14 to liver volume on POD 0 were inversely correlated with remnant liver volume on POD 0 ($r = -0.91$). The ratio of liver volume on POD 14 to liver volume on POD 0 were significantly correlated with serum hepatocyte growth factor (HGF) levels on POD 1 ($r = 0.54$), serum leptin levels on POD 1 ($r = 0.54$), and serum macrophage colony-stimulating

factor (M-CSF) levels on POD 5 ($r = 0.76$) and POD 7 ($r = 0.80$). These results suggest that early-phase elevation of serum levels of HGF, leptin and M-CSF may be associated with the acceleration of liver regeneration after hepatectomy in humans.

Keywords: hepatectomy; hepatocyte growth factor; human; leptin; liver regeneration; macrophage colony-stimulating factor

1. Introduction

Liver transplantation is the only curative treatment for end-stage liver diseases. However, in a setting of the shortage of liver grafts, many patients deteriorate as a result of disease progression or develop complications because of the lack of a timely suitable donor while waiting for a liver graft [1,2]. Thus, in addition to liver transplantation, new therapeutic agents for promoting liver regeneration are desired.

In animal models, the mechanisms of liver regeneration have been investigated in detail. Hepatocytes are primed by tumor necrosis factor (TNF)-α and interleukin (IL)-6 mainly produced by Kupffer cells, and then proliferation and cell growth of hepatocytes are induced in response to transforming growth factor-α, hepatocyte growth factor (HGF), and epidermal growth factor [3]. In addition, vascular endothelial growth factor (VEGF) and thrombopoietin (TPO) are shown to promote liver regeneration [4,5].

In humans, *in vivo* investigations of liver regeneration have been mainly performed in patients undergoing surgical resection of liver cancers or liver transplant recipients; however, underlying diseases and immunosuppressant after liver transplantation may influence liver regeneration. Until now, a few studies have shown that serum HGF and IL-6 levels are elevated on postoperative day (POD) 1 [6–8]. In individuals without the appropriate elevation of serum HGF levels after partial hepatectomy, postoperative liver failure develops more frequently [9]. Serial changes of serum VEGF and TPO levels after partial hepatectomy have been also investigated in healthy liver donors [7,10]. However, associations of these growth factors with liver regeneration have not been fully revealed.

Recently, because of the shortage of liver grafts from deceased donors, the number of living donor liver transplantation has increased. In living donor liver transplantation, healthy liver donors undergo typical and anatomical hepatectomy. So, the mechanisms of liver regeneration in healthy humans, which may be different from those in patients undergoing surgical resection of liver cancers, liver transplant recipients, and animal models, may be revealed. In this study, we investigated the serial changes of serum levels of various growth factors after partial hepatectomy and the associations of these changes of various growth factors with liver regeneration after the operation in healthy liver donors.

2. Results

2.1. Clinical Characteristics of Study Population

Clinical characteristics of 16 healthy liver donors are shown in Table 1. Preoperative liver function tests were within normal limit in all patients. Each donor did not require perioperative transfusion or suffer from any major operative complications after surgery.

Liver graft type and changes of liver volume before and after partial hepatectomy are summarized in Table 2. The ratio of remnant liver volume on POD 0 to liver volume before the operation was 51% ± 20%. The ratio of liver volume on POD 14 to liver volume before the operation was 76% ± 11%. Remnant liver volume per body weight on POD 0 were more in left graft donors than in right graft donors (15.6 ± 1.8 cm^3/kg *versus* 7.7 ± 2.7 cm^3/kg, $p < 0.0001$); however, the ratio of liver volume on POD 14 to liver volume on POD 0 was higher in right liver donors than in left liver donors (199% ± 42% *versus* 114% ± 8%, $p = 0.0003$). Ratio of liver volume on POD 14 to liver volume on POD 0 was inversely correlated with remnant liver volume on POD 0 ($r = -0.91$, $p < 0.0001$) and remnant liver volume per body weight on POD 0 ($r = -0.95$, $p < 0.0001$). On the other hand, the ratio of liver volume on POD 14 to liver volume on POD 0 was not associated with gender, age and body mass index.

Table 1. Clinical characteristics of 16 healthy liver donors on admission.

Clinical Characteristics	Value
Age (year)	36 ± 12
Gender, female (%)	12 (75)
Height (cm)	161 ± 6
Body weight (kg)	59 ± 11
Body mass index (kg/m^2)	22.8 ± 4.2
Laboratory Data	**Value**
White blood cell count ($/mm^3$)	5574 ± 890
Hemoglobin concentration (g/dL)	13.4 ± 1.8
Platelet count ($\times 10^4/mm^3$)	24.7 ± 3.9
Bilirubin (mg/dL)	0.9 ± 0.5
Albumin (g/dL)	4.5 ± 0.3
Prothrombin time-international normalized ratio (INR)	0.98 ± 0.07
Aspartate aminotransferase (IU/L)	18 ± 4
Alanine aminotransferase (IU/L)	15 ± 9
C-reactive protein (mg/dL)	0.05 ± 0.07

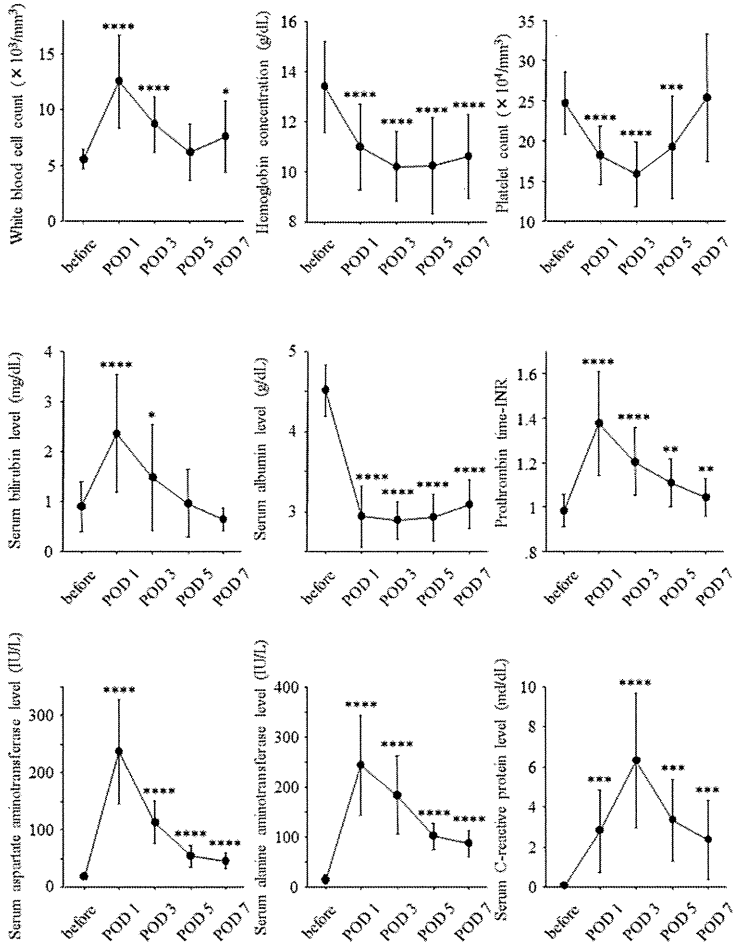
Table 2. Liver graft type and changes of liver volume before and after hepatectomy.

Graft Type	Value
Liver graft type (left graft) n (%)	6 (38)
Liver graft type (right graft) n (%)	10 (62)
Liver volume Change	**Value**
Liver volume before hepatectomy (cm^3)	1213 ± 206
Liver resection rate (%)	49 ± 20
Remnant liver volume on POD 0 (cm^3)	622 ± 262
Remnant liver volume per body weight on POD 0 (cm^3/kg)	10.7 ± 4.6
Liver volume on POD 14 (cm^3)	917 ± 158
Ratio of liver volume on POD 14 to liver volume on POD 0 (%)	167 ± 54

2.2. Postoperative Changes of Laboratory Data and Liver Regeneration

Serial changes of laboratory data before hepatectomy and on POD 1, 3, 5 and 7 are shown in Figure 1.

Figure 1. Serial changes of laboratory data during the clinical course. Laboratory data before hepatectomy and on postoperative day (POD) 1, 3, 5 and 7 were expressed as mean ± standard deviation. Before: before partial hepatectomy; POD: postoperative day; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

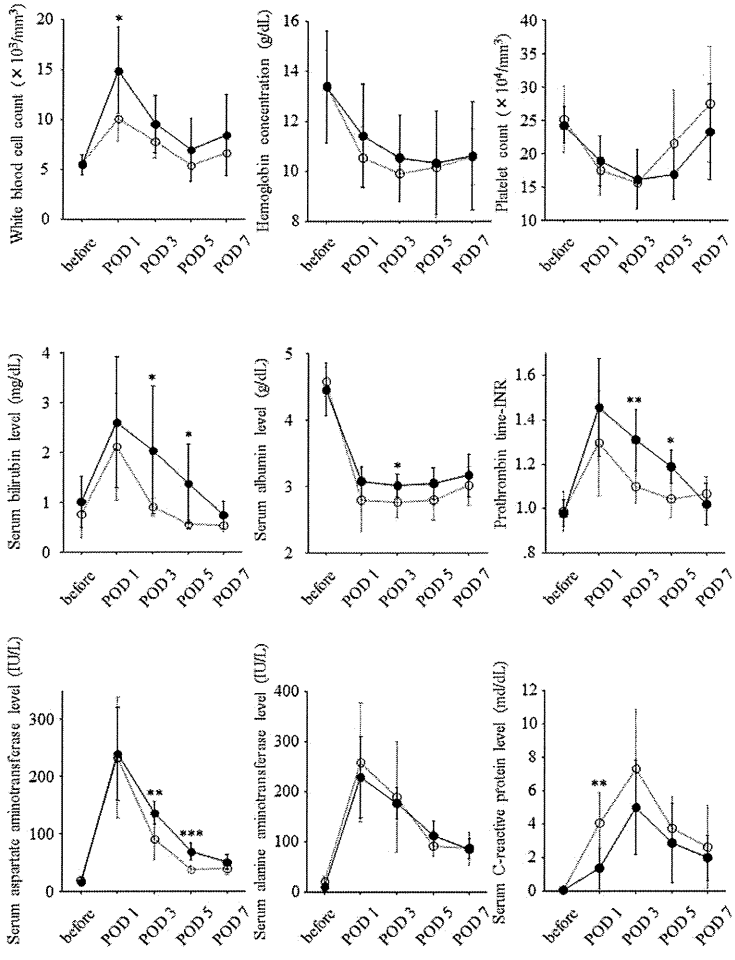


Liver resection rate was significantly correlated with white blood cell counts on POD 1 ($r = 0.65$, $p = 0.005$), serum bilirubin levels on POD 3 ($r = 0.51$, $p = 0.045$), serum albumin levels on POD 3 ($r = 0.57$, $p = 0.020$), serum aspartate aminotransferase levels on POD 3 ($r = 0.63$, $p = 0.007$) and POD 5 ($r = 0.81$, $p = 0.0006$), and prothrombin time-international normalized ratio (INR) on POD 3 ($r = 0.71$, $p = 0.002$) and POD 5 ($r = 0.78$, $p = 0.004$) but was inversely correlated with serum *C*-reactive protein levels ($r = -0.67$, $p = 0.005$). Remnant liver volume per body weight on POD 0 was inversely correlated with white blood cell counts on POD 1 ($r = -0.61$, $p = 0.011$), serum aspartate aminotransferase levels on POD 3 ($r = -0.78$, $p = 0.0002$) and POD 5 ($r = -0.78$, $p = 0.019$), and prothrombin time-INR on POD 3 ($r = -0.68$, $p = 0.003$) and POD 5 ($r = -0.78$, $p = 0.003$) but was significantly correlated with serum C-reactive protein levels ($r = 0.66$, $p = 0.006$).

According to remnant liver volume per body weight on POD 0, 16 patients were divided into two groups. One group consisted of eight patients with remnant liver volume per body weight on POD 0 of 10 cm^3/kg or less, and another group consisted of the other eight patients with remnant liver volume per body weight on POD 0 >10 cm^3/kg. Serial changes of laboratory data in both the groups

are shown in Figure 2. White blood cell counts on POD 1, serum bilirubin levels on POD 3 and 5, serum albumin levels on POD 3, serum aspartate aminotransferase levels on POD 3 and 5, and prothrombin time-INR on POD 3 and 5 were significantly higher in the eight patients with remnant liver volume per body weight on POD 0 of 10 cm^3/kg or less. On the other hand, serum C-reactive protein levels on POD 1 were lower in this group.

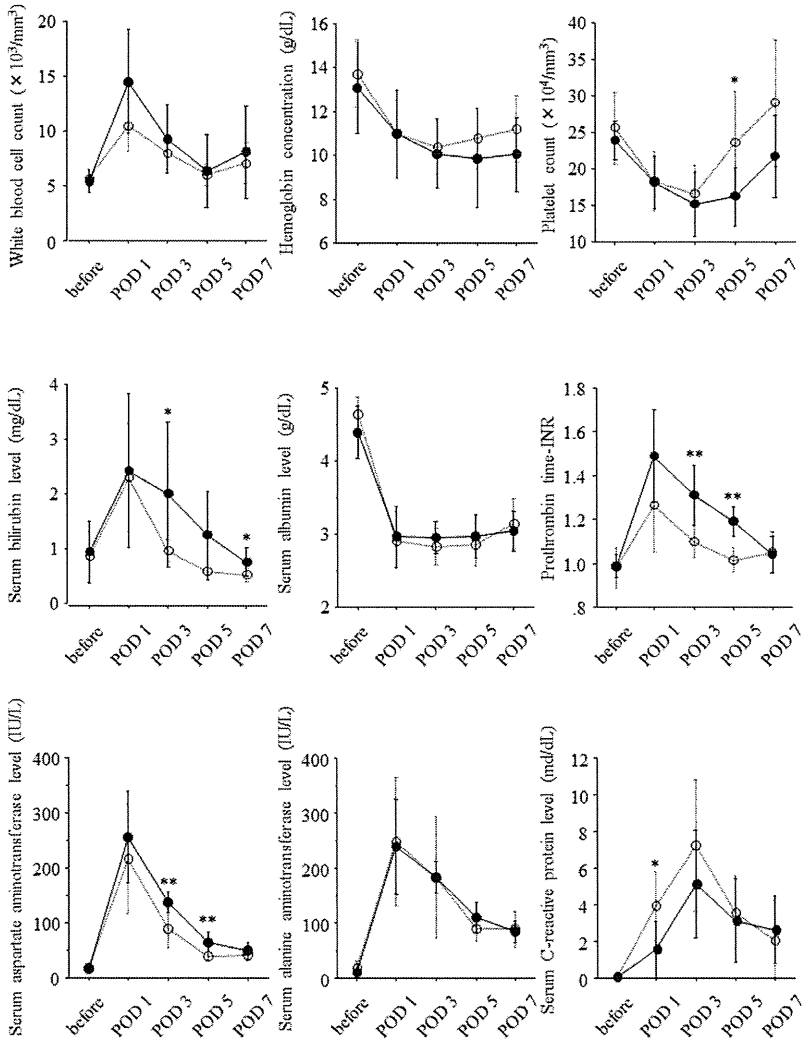
Figure 2. Associations of remnant liver volume per body weight on POD 0 with serial changes of laboratory data during the clinical course. Solid and dotted lines show serial changes of serum levels of each growth factor in eight patients with remnant liver volume per body weight on POD 0 of 10 cm^3/kg or less and the other eight patients with remnant liver volume per body weight on POD 0 > 10 cm^3/kg, respectively. Serum levels of each growth factor before hepatectomy and on POD 1, 3, 5 and 7 were expressed as mean ± standard deviation. Before: before partial hepatectomy; POD: postoperative day; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.



Ratio of liver volume on POD 14 to liver volume on POD 0 was correlated with white blood cell counts on POD 1 ($r = 0.63$, $p = 0.007$), prothrombin time-INR on POD 3 ($r = 0.62$, $p = 0.009$) and POD 5 ($r = 0.72$, $p = 0.010$), and serum aspartate aminotransferase levels on POD 3 ($r = 0.71$, $p = 0.002$) and POD 5 ($r = 0.67$, $p = 0.015$). On the other hand, serum C-reactive protein levels on POD 1 were inversely correlated with ratio of liver volume on POD 14 to liver volume on POD 0 ($r = -0.62$, $p = 0.012$).

According to the ratio of liver volume on POD 14 to liver volume on POD 0, 16 patients were divided into two groups. Eight patients showing ratio of liver volume on POD 14 to liver volume on POD 0 of 150% or higher were classified into high liver regeneration group, and the others eight showing this ratio <150% were classified into low liver regeneration group. Serial changes of laboratory data in both the groups are shown in Figure 3. Prothrombin time-INR on POD 3 and 5, serum bilirubin levels on POD 3 and 7, and serum aspartate aminotransferase levels on POD 3 and 5 were significantly higher in high liver regeneration group. On the other hand, platelet counts on POD 5 and serum *C*-reactive protein levels on POD 1 were lower in the high liver regeneration group.

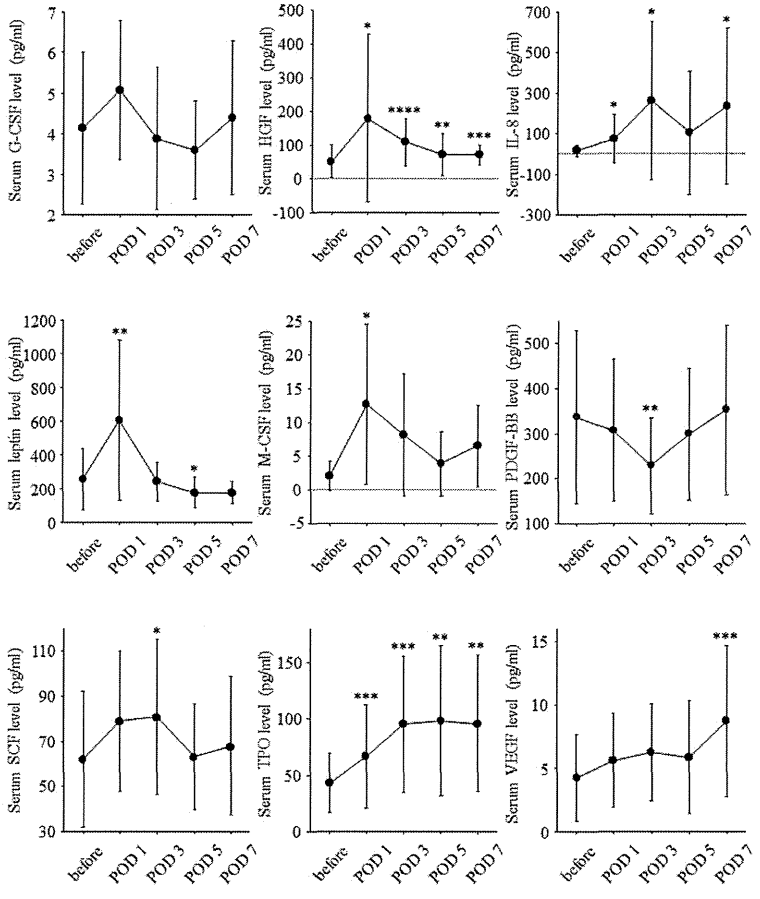
Figure 3. Associations of liver regeneration with serial changes of laboratory data during the clinical course. Solid and dotted lines show serial changes of laboratory data in eight patients showing ratio of liver volume on POD 14 to liver volume on POD 0 of 150% or higher and the other eight patients showing this ratio <150%, respectively. Serum levels of each laboratory data before hepatectomy and on POD 1, 3, 5 and 7 were expressed as mean ± standard deviation. Before: before partial hepatectomy; POD: postoperative day; *: $p < 0.05$; **: $p < 0.01$.



2.3. Postoperative Changes of Serum Growth Factor Levels and Liver Regeneration

Serial changes of serum growth factor levels are shown in Figure 4. Postoperative changes in serum levels of HGF and leptin paralleled those in prothrombin time-INR and serum levels of bilirubin. The changes in serum levels of macrophage colony-stimulating factor (M-CSF) paralleled those in white blood cell counts. The changes in serum platelet-derived growth factor (PDGF)-BB levels paralleled those in platelet counts.

Figure 4. Serial changes of serum levels of nine growth factors during the clinical course. Serum levels of each growth factor before hepatectomy and on POD 1, 3, 5 and 7 were expressed as mean ± standard deviation. Before: before partial hepatectomy; POD: postoperative day; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$; ****: $p < 0.0001$.

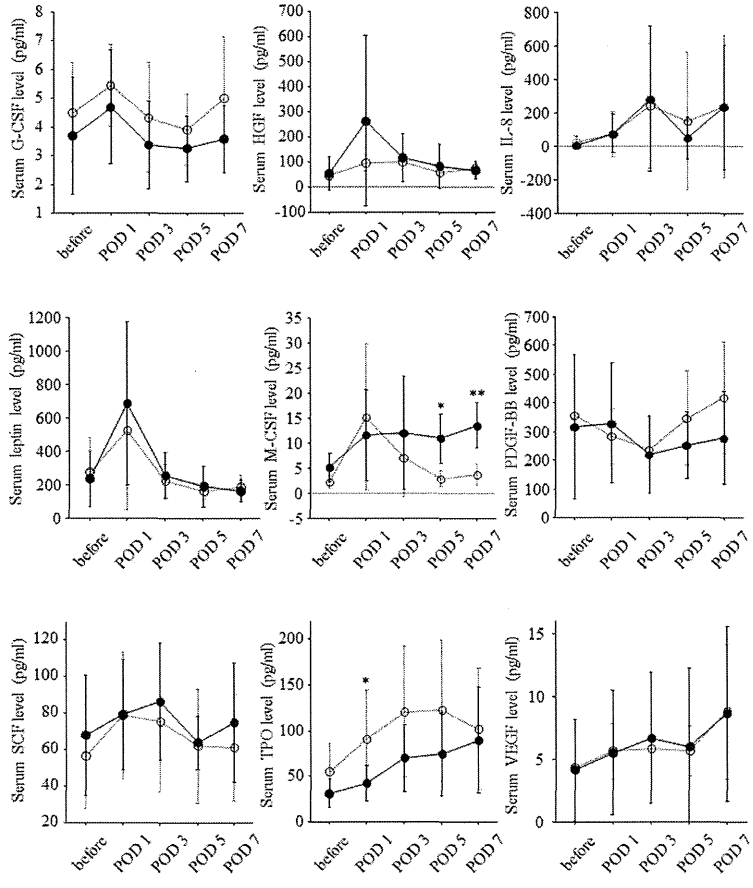


Liver resection rate was significantly correlated with serum M-CSF levels on POD 5 (r = 0.78, p = 0.037) and POD 7 (r = 0.81, p = 0.003) but not with serum HGF and leptin levels on POD 1. Remnant liver volume per body weight on POD 0 was inversely correlated with serum M-CSF levels on POD 5 (r = −0.76, p = 0.045) and POD 7 (r = −0.75, p = 0.010) and tended to be inversely correlated with serum HGF levels on POD 1 (r = −0.46, p = 0.076) and serum leptin levels on POD 1 ($r = -0.47, p = 0.064$).

According to remnant liver volume per body weight on POD 0, serial changes of serum growth factor levels are shown in Figure 5. In eight patients with remnant liver volume per body weight on

POD 0 of 10 cm^3/kg or less, serum M-CSF levels on POD 5 and POD 7 were significantly higher. On the other hand, serum TPO levels on POD 1 were lower in this group.

Figure 5. Associations of remnant liver volume per body weight on POD 0 with serial changes of serum levels of nine growth factors during the clinical course. Solid and dotted lines show serial changes of serum levels of each growth factor in eight patients with remnant liver volume per body weight on POD 0 of 10 cm^3/kg or less and the other eight patients with remnant liver volume per body weight on POD 0 >10 cm^3/kg, respectively. Serum levels of each growth factor before hepatectomy and on POD 1, 3, 5 and 7 were expressed as mean ± standard deviation. Before: before partial hepatectomy; POD: postoperative day; *: $p < 0.05$; **: $p < 0.01$.

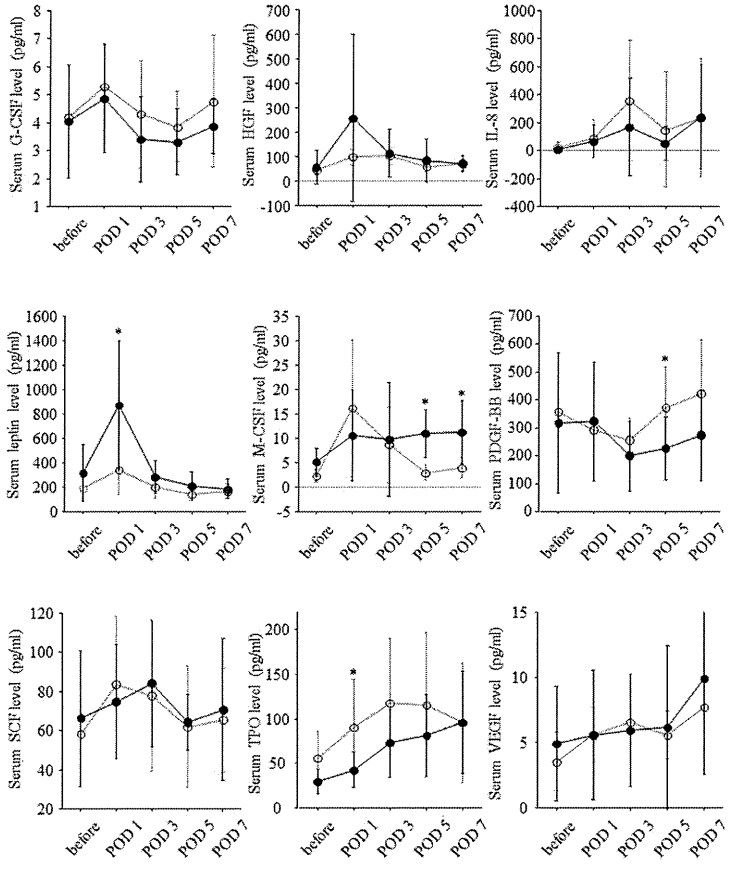


Ratio of liver volume on POD 14 to liver volume on POD 0 was significantly correlated with serum HGF levels on POD 1 ($r = 0.54$, $p = 0.030$), serum leptin levels on POD 1 ($r = 0.54$, $p = 0.028$), and serum M-CSF levels on POD 5 ($r = 0.76$, $p = 0.047$) and POD 7 ($r = 0.80$, $p = 0.003$). On the other hand, ratio of liver volume on POD 14 to liver volume on POD 0 was inversely correlated with serum PDGF-BB levels on POD 5 ($r = -0.61$, $p = 0.011$), and serum TPO levels on POD 1 ($r = -0.60$, $p = 0.012$).

Serial changes of serum growth factor levels in high liver regeneration group and low liver regeneration group are shown in Figure 6. Serum leptin levels on POD 1 and serum M-CSF levels on POD 5 and POD 7 were significantly higher in high liver regeneration group. Serum HGF levels on POD 1 seemed to be higher in high liver regeneration group although the difference was not

significant. On the other hand, serum PDGF-BB levels on POD 5 and serum TPO levels on POD 1 were lower in the high liver regeneration group.

Figure 6. Associations of liver regeneration with serial changes of serum levels of nine growth factors during the clinical course. Solid and dotted lines show serial changes of serum levels of each growth factor in eight patients showing ratio of liver volume on POD 14 to liver volume on POD 0 of 150% or higher and the other eight patients showing this ratio <150%, respectively. Serum levels of each growth factor before hepatectomy and on POD 1, 3, 5 and 7 were expressed as mean ± standard deviation. Before: before partial hepatectomy; POD: postoperative day; *: $p < 0.05$.



3. Discussion

The liver has strong potential to regenerate. Liver regeneration involves a complex interaction of the proliferation of resident hepatocytes and hepatocyte progenitor cells, the facilitation of angiogenesis, and the differentiation of hematopoietic stem cells into hepatocyte. However, the mechanism of liver regeneration in healthy humans has not been revealed yet. This study indicated that, after partial hepatectomy of the grade not exerting danger on a life, the smaller the remnant liver volume, the higher was liver regeneration, and that various growth factors intricately took parts in liver regeneration after partial hepatectomy. In particular, early-phase elevations of serum levels of HGF, leptin and M-CSF seemed to be associated with the acceleration of liver regeneration after partial hepatectomy.

As is well known, HGF is a potent factor for proliferation of hepatocyte. In this study, serum HGF levels on POD 1 were correlated with ratio of liver volume on POD 14 to liver volume on POD 0. These findings are consistent with the previous reports [6,7]. Recently, a clinical trial using recombinant HGF for acute liver failure has been reported, and it has been shown that intravenous administration of recombinant HGF is well-tolerated [11]. Further clinical trials are required to determine the effect of recombinant HGF on liver regeneration in humans.

Some studies have showed the relation of leptin with liver regeneration in animal models. In leptin-deficient ob/ob mice after toxic liver injury or partial hepatectomy, liver regeneration is impaired with down-regulated hepatic expression of TNF-α and IL-6, and leptin supplementation improves liver regeneration with up-regulated hepatic expression of TNF-α and IL-6 [12,13]. On the other hand, leptin does not directly up-regulate hepatocyte proliferation [14]. Leptin may accelerate liver regeneration through the release of cytokines such as TNF-α and IL-6 from non-parenchymal cells.

M-CSF is produced by non-parenchymal and parenchymal liver cells. In M-CSF-deficient mice, hepatic expressions of TNF-α and IL-6 are reduced, and proliferation of hepatocytes is impaired [15]. On the other hand, in M-CSF-deficient mice, M-CSF supplementation improves liver regeneration [15]. In addition, hepatocyte-like cells are reported to differentiate from peripheral blood monocytes under the stimulation of M-CSF [16]. M-CSF may take a part in liver regeneration through the proliferation of hepatocytes and the differentiation of hematopoietic stem cells into hepatocytes.

An appropriate intra-hepatic inflammatory response to liver injury has been shown to promote liver regeneration [17,18]. In this study, white blood cell counts on POD 1 were correlated with ratio of liver volume on POD 14 to liver volume on POD 0. However, serum *C*-reactive protein levels on POD 1 were shown to be inversely correlated with ratio of liver volume on POD 14 to liver volume on POD 0. This may be partially due to the interaction of *C*-reactive protein with leptin. *C*-reactive protein is reported to inhibit the binding of leptin to its receptor and attenuate its physiological functions [19]. In addition, *C*-reactive protein are shown to induce hepatic insulin-resistance which leads to poor liver regeneration [20,21].

Serum TPO levels in this study were gradually increased after partial hepatectomy, and these changes are consistent with the previous report [10]. TPO promotes liver regeneration after partial hepatectomy [5]. However, in this study, serum TPO levels on POD 1 were correlated with remnant liver volumes on POD 0. TPO is mainly produced by hepatocyte in response to thrombocytopenia when circulating platelet counts is decreased [22]. In this study, platelet counts abruptly decreased after the operation. In response to thrombocytopenia, serum TPO levels after the operation may be elevated in proportion to remnant liver volumes.

4. Materials and Methods

This study was approved by the Institutional Review Board at Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama, Japan. Each patient was informed of the nature of the study and signed an informed consent form.

4.1. Study Population

Sixteen healthy liver donors who underwent partial hepatectomy between January 2000 and November 2010 were prospectively included in this study. Eight donors underwent a right lobectomy, three did an extended left lobectomy, two did a left lateral segmentectomy, one did a left lobectomy, and two did a right posterior segmentectomy, respectively.

4.2. Measurement of Serum Growth Factor Level

Sera were collected prior to the operation and on POD 1, 3, 5 and 7. Samples were frozen and stored at −80 °C until analysis.

Serum levels of the following growth factors were measured using the Bio-Plex Protein Array System (Bio-Rad Laboratories, Hercules, CA, USA): granulocyte colony-stimulating factor, HGF, IL-8, leptin, M-CSF, PDGF-BB, stem cell factor, and VEGF. In brief, the Bio-Plex Pro Standard and samples diluted in Serum Diluent were added to a 96-well filter plate and incubated with the antibody-coupled beads for 1 h with continuous shaking. The beads were washed three times with wash buffer to remove unbound protein and incubated with biotinylated detection antibodies for 30 min with continuous shaking. Following three washes, premixed streptavidin-phycoerythrin was added to each well and incubated for 30 min. After incubation, the beads were washed and re-suspended in assay buffer. The reaction mixture was quantified using the Bio-Plex protein array reader. Each growth factor level was automatically calculated by Bio-Plex Manager software using the appropriate standard curve.

Serum TPO level was measured using an enzyme-linked immunosorbent assay kit according to the manufacturer's instructions (Quantikine Human TPO Immunoassay, R&D Systems, Minneapolis, MN, USA). Microplates were coated with manufacturer-provided monoclonal antibodies against TPO, and following the enzyme reaction the plates were measured using a microplate manager (BIO-RAD Laboratories, Hercules, CA, USA) and the optical density was determined at 450 nm.

4.3. Volumetric Study of Liver

Liver volumes were measured by multi-detector computed tomography (Aquilion 64, Toshiba Medical Systems Corporation, Otowara, Japan) using workstation (Virtual Place Advance Plus, Aze, Tokyo, Japan).

The liver resection rate (%) was calculated as follows: resected liver graft volume (cm^3)/liver volume before the operation (cm^3) × 100%.

4.4. Statistical Analysis

SPSS statistical program (release 11.0.1 J, SPSS, Chicago, IL, USA) was used for the statistical analysis.

Dichotomous variables were compared by the chi-squared test. Continuous variables were expressed as mean ± standard deviation (SD). Student's *t*-test was used to evaluate differences in the continuous variables between two groups. The Pearson's correlation test was used to evaluate the consistency in the continuous variables between two groups. p-values < 0.05 were considered significant.

5. Conclusions

After partial hepatectomy of the grade not exerting danger on a life, the smaller the remnant liver volume, the higher the liver regeneration is. This study indicates that various growth factors are associated with liver regeneration after partial hepatectomy in healthy humans. In particular, early-phase elevation of serum levels of HGF, leptin and M-CSF may be associated with accelerated liver regeneration. HGF, leptin and M-CSF possibly become new therapeutic agents for promoting liver regeneration. In addition, serial changes of serum levels of these growth factors may be early predictors of liver regeneration after hepatectomy. In order to confirm these findings in healthy humans, further studies are required.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Merion, R.M. Current status and future of liver transplantation. *Semin. Liver Dis.* **2010**, *30*, 411–421.
2. Li, K.K.; Neuberger, J. The management of patients awaiting liver transplantation. *Nat. Rev. Gastroenterol. Hepatol.* **2009**, *6*, 648–659.
3. Fausto, N.; Campbell, J.S.; Riehle, K.J. Liver regeneration. *Hepatology* **2006**, *43*, S45–S53.
4. Assy, N.; Spira, G.; Paizi, M.; Shenkar, L.; Kraizer, Y.; Cohen, T.; Neufeld, G.; Dabbah, B.; Enat, R.; Baruch, Y. Effect of vascular endothelial growth factor on hepatic regenerative activity following partial hepatectomy in rats. *J. Hepatol.* **1999**, *30*, 911–915.
5. Murata, S.; Hashimoto, I.; Nakano, Y.; Myronovych, A.; Watanabe, M.; Ohkohchi, N. Single administration of thrombopoietin prevents progression of liver fibrosis and promotes liver regeneration after partial hepatectomy in cirrhotic rats. *Ann. Surg.* **2008**, *248*, 821–828.
6. De Jong, K.P.; von Geusau, B.A.; Rottier, C.A.; Bijzet, J.; Limburg, P.C.; de Vries, E.G.; Fidler, V.; Slooff, M.J. Serum response of hepatocyte growth factor, insulin-like growth factor-I, interleukin-6, and acute phase proteins in patients with colorectal liver metastases treated with partial hepatectomy or cryosurgery. *J. Hepatol.* **2001**, *34*, 422–427.
7. Efimova, E.A.; Glanemann, M.; Nussler, A.K.; Schumacher, G.; Settmacher, U.; Jonas, S.; Nussler, N.; Neuhaus, P. Changes in serum levels of growth factors in healthy individuals after living related liver donation. *Transplant. Proc.* **2005**, *37*, 1074–1075.
8. Nakashima, S.; Katano, Y.; Nakano, I.; Hirooka, Y.; Ito, A.; Ishigami, M.; Hayashi, K.; Honda, T.; Goto, H. Changes in circulating cytokine levels and lymphocyte subsets in healthy liver donors after partial hepatectomy. *Hepatol. Res.* **2007**, *37*, 878–884.
9. Nishizaki, T.; Takenaka, K.; Yoshizumi, T.; Yanaga, K.; Soejima, Y.; Shirabe, K.; Sugimachi, K. Alteration in levels of human hepatocyte growth factor following hepatectomy. *J. Am. Coll. Surg.* **1995**, *181*, 6–10.
10. Nagasako, Y.; Jin, M.B.; Miyazaki, H.; Nakayama, M.; Shimamura, T.; Furukawa, H.; Matushita, M.; Todo, S. Thrombopoietin in postoperative thrombocytopenia following living donor hepatectomy. *Liver Transplant.* **2006**, *12*, 435–439.

11. Ido, A.; Moriuchi, A.; Numata, M.; Murayama, T.; Teramukai, S.; Marusawa, H.; Yamaji, N.; Setoyama, H.; Kim, D., II; Chiba, T.; *et al.* Safety and pharmacokinetics of recombinant human hepatocyte growth factor (rh-HGF) in patients with fulminant hepatitis: A phase I/II clinical trial, following preclinical studies to ensure safety. *J. Transl. Med.* **2011**, *9*, 55.
12. Leclercq, I.A.; Field, J.; Farrell, G.C. Leptin-specific mechanisms for impaired liver regeneration in *ob*/*ob* mice after toxic injury. *Gastroenterology* **2003**, *124*, 1451–1464.
13. Leclercq, I.A.; Vansteenberghe, M.; Lebrun, V.B.; VanHul, N.K.; Abarca-Quinones, J.; Sempoux, C.L.; Picard, C.; Stärkel, P.; Horsmans, Y.L. Defective hepatic regeneration after partial hepatectomy in leptin-deficient mice is not rescued by exogenous leptin. *Lab. Investig.* **2006**, *86*, 1161–1171.
14. Yang, S.; Koteish, A.; Lin, H.; Huang, J.; Roskams, T.; Dawson, V.; Diehl, A.M. Oval cells compensate for damage and replicative senescence of mature hepatocytes in mice with fatty liver disease. *Hepatology* **2004**, *39*, 403–411.
15. Amemiya, H.; Kono, H.; Fujii, H. Liver regeneration is impaired in macrophage colony stimulating factor deficient mice after partial hepatectomy: The role of M-CSF-induced macrophages. *J. Surg. Res.* **2011**, *165*, 59–67.
16. Ruhnke, M.; Ungefroren, H.; Nussler, A.; Martin, F.; Brulport, M.; Schormann, W.; Hengstler, J.G.; Klapper, W.; Ulrichs, K.; Hutchinson, J.A.; *et al.* Differentiation of *in vitro*-modified human peripheral blood monocytes into hepatocyte-like and pancreatic islet-like cells. *Gastroenterology* **2005**, *128*, 1774–1786.
17. Ohnishi, T.; Kakimoto, K.; Bandow, K.; Lowenstein, C.J.; Daikuhara, Y.; Matsuguchi, T. Mature hepatocyte growth factor/scatter factor on the surface of human granulocytes is released by a mechanism involving activated factor Xa. *J. Immunol.* **2006**, *176*, 6945–6953.
18. Viebahn, C.S.; Benseler, V.; Holz, L.E.; Elsegood, C.L.; Vo, M.; Bertolino, P.; Ganss, R.; Yeoh, G.C. Invading macrophages play a major role in the liver progenitor cell response to chronic liver injury. *J. Hepatol.* **2010**, *53*, 500–507.
19. Chen, K.; Li, F.; Li, J.; Cai, H.; Strom, S.; Bisello, A.; Kelley, D.E.; Friedman-Einat, M.; Skibinski, G.A.; McCrory, M.A.; *et al.* Induction of leptin resistance through direct interaction of C-reactive protein with leptin. *Nat. Med.* **2006**, *12*, 425–432.
20. Aoyama, T.; Ikejima, K.; Kon, K.; Okumura, K.; Arai, K.; Watanabe, S. Pioglitazone promotes survival and prevents hepatic regeneration failure after partial hepatectomy in obese and diabetic KK-A^y mice. *Hepatology* **2009**, *49*, 1636–1644.
21. Xi, L.; Xiao, C.; Bandsma, R.H.J.; Naples, M.; Adeli, K.; Lewis, G.F. *C*-reactive protein impairs hepatic insulin sensitivity and insulin signaling in rats: Role of mitogen-activated protein kinases. *Hepatology* **2011**, *53*, 127–135.
22. Afdhal, N.; McHutchison, J.; Brown, R.; Jacobson, I.; Manns, M.; Poordad, F.; Weksler, B.; Esteban, R. Thrombocytopenia associated with chronic liver disease. *J. Hepatol.* **2008**, *48*, 1000–1007.

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ORIGINAL ARTICLE

Risk factors for acute renal injury in living donor liver transplantation: evaluation of the RIFLE criteria

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acute renal failure, liver transplantation, living donor, RIFLE criteria.

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Summary

Acute renal injury (ARI) is a serious complication after liver transplantation. This study investigated the usefulness of the RIFLE criteria in living donor liver transplantation (LDLT) and the prognostic impact of ARI after LDLT. We analyzed 200 consecutive adult LDLT patients, categorized as risk (R), injury (I), or failure (F), according to the RIFLE criteria. ARI occurred in 60.5% of patients: R-class, 23.5%; I-class, 21%; and F-class, 16%. Four patients in Group-A (normal renal function and R-class) and 26 patients in Group-B (severe ARI: I- and F-class) required renal replacement therapy ($P < 0.001$). Mild ARI did not affect postoperative prognosis regarding hospital mortality rate in Group A (3.2%), which was superior to that in Group B (15.8%; $P = 0.0015$). Fourteen patients in Group B developed chronic kidney disease (KDIGO stage 3/4). The 1-, 5- and 10-year survival rates were 96.7%, 90.6%, and 88.1% for Group A and 71.1%, 65.9%, and 59.3% for Group B, respectively ($P < 0.0001$). Multivariate analysis revealed risk factors for severe ARI as MELD ≥20 [odds ratio (OR) 2.9], small-for-size graft (GW/RBW <0.7%; OR 3.1), blood loss/body weight >55 ml/kg (OR 3.7), overexposure to calcineurin inhibitor (OR 2.5), and preoperative diabetes mellitus (OR 3.2). The RIFLE criteria offer a useful predictive tool after LDLT. Severe ARI, defined beyond class-I, could have negative prognostic impact in the acute and late postoperative phases. Perioperative treatment strategies should be designed and balanced based on the risk factors for the further improvement of transplant prognosis.

Introduction

Acute renal injury (ARI) is a serious complication after liver transplantation. Several studies have demonstrated an association between ARI and increased mortality rates after cadaveric liver transplantation [1–3]. The incidence of postliver transplant ARI has been reported with a wide range in the literature, because of the use of different definitions and parameters [4–8]. Until recently, more than 30 different definitions of ARI have been used in the literature. This lack of common reference points has created confusion and complicated the interpretation of findings. It has also led to strong advocacy for a consensus definition. In response to the need for common definitions and classifications of ARI, the Acute Dialysis Quality Initiative group of experts (http://www.adqi.net) developed a consensus definition for ARI in critically ill patients (the RIFLE criteria) based on changes in glomerular filtration rate (GFR) and/or urine output. RIFLE is an acronym for "risk of renal dysfunction, injury to the kidney, failure of the kidney, loss of the kidney and end-stage kidney disease" [9]. These criteria have been evaluated in several studies, showing that acute kidney disease is associated with significantly higher mortality rates [10–12]. Several studies have also demonstrated that ARI is associated with the development of chronic kidney disease (CKD) [13,14].

These criteria can be suitable for cadaveric liver transplantation [13,15,16]. In living donor liver transplantation (LDLT), graft size seems to be an indispensable factor for predicting post-transplant ARI and prognosis, in addition to the conventional risk factors [17]. Despite the important implications of the RIFLE criteria for cadaveric liver transplantation, no studies have yet dealt with LDLT; however, the RIFLE criteria are also expected to serve as a useful prognostic predictor after LDLT. The aim of this study was to clarify the usefulness of the RIFLE criteria in LDLT and to determine risk factors for ARI after LDLT. This study also focused on evaluating the relationship between ARI and post-transplant mortality, the influence of ARI on CKD, and late postoperative phase prognosis.

Materials and methods

Patients

In this retrospective analysis, we reviewed 200 consecutive adult patients undergoing LDLT at Okayama University Hospital between August 1996 and January 2011. The study subjects comprised 57.8% men (overall mean age, 49.2 ± 11.8 years). Indications for LDLT in these patients included postnecrotic liver cirrhosis (n = 126; 63%), cholestatic disease (n = 39; 19.5%), acute liver failure (n = 24; 11.9%), and metabolic disorder (n = 11; 5.5%). Among the patients with postnecrotic liver cirrhosis, hepatitis C virus (HCV) was the predominant etiology (n = 62; 49.2%). Hepatocellular carcinoma (HCC) accounted for 48.4% (n = 61) of all cirrhotic patients.

In terms of surgical technique and postoperative care, the procedures and protocols were followed as described previously, with minor modifications [18–21]. In the donor procedure, parenchymal dissection was performed without hepatic inflow occlusion, followed by graft procurement. In the recipient procedure, the native liver was resected, preserving the inferior vena cava. After reconstructing the hepatic and portal veins, the hepatic artery was anastomosed under microscopy. The biliary tract was reconstructed. During the postoperative period, the initial immunosuppressive regimen consisted of tacrolimus or cyclosporine and a short course of steroids, tapering over 3–6 months. The dosage was carefully adjusted according to the drug trough level, targeting trough levels of 10–12 ng/ml for tacrolimus and 150–200 ng/ml for cyclosporine. Whole-blood tacrolimus or cyclosporine drug trough levels were measured at 12 h after administration of the drug during the postoperative acute phase. Averaged calcineurin inhibitor (CNI) trough level represented the whole blood concentration within the first month or prior to develop ARI. The measurement protocol for CNI which had undergone the following changes is now affinity column-mediated immunoassay method. During the period between 1998 and 2003, both agents were measured by enzyme-linked immunosorbent assay method which was substituted by microparticle enzyme immunoassay method in tacrolimus and by monoclonal fluorescence polarization immunoassay method up to 2008. Concerning measurement protocol for CNI, new measurement technologies have been developed within the study period. In this study, the historical bias between the measurement protocols could seem to be allowable [22–26]. We introduced mycophenolate mofetil (MMF) in August 2002 and used MMF for every patient for initial immunosuppression. The main purpose of the MMF was to diminish the CNI dosage and lower the CNI trough levels to avoid any adverse events related to CNI. MMF was administered to some patients in whom the trough levels of CNI diminished to 70–80%. In our protocol, MMF is started from 5 to 7 days after LDLT. In cases of ARI, early renal replacement therapy (RRT) was introduced as support until the kidneys recovered function. The choice of intermittent hemodialysis or continuous RRT was based on the hemodynamic stability of the patient.

All 200 LDLT recipients were classified according to these RIFLE criteria using the worst value of renal function within 28 days after LDLT. Because classes L and E should be used to denote persistent disease for more than 4 weeks, all patients were classified in classes R to F rather than classes L or E in this study. After follow-up for 1 year following LDLT, patients with persistent chronic kidney dysfunction were classified according to the KDIGO Clinical Practice Guidelines as CKD stage 3 if the GFR was 30–59 ml/min; CKD stage 4 if the GFR was 15–29 ml/min; and CKD stage 5 if GFR was <15 ml/min or dialysis, depending on the last value of the GFR [27,28].

Statistical analysis

Nonparametric methods were used for inferential analysis. Continuous variables were evaluated using the Mann–Whitney test, and categorical data were compared by the chi-squared test. Overall survival rates were estimated by the Kaplan–Meier method and compared using the log-rank test. Sixteen clinical variables potentially associated with the occurrence of severe ARI were adopted for multivariate logistic regression analysis, after employment of cut-off values for continuous variables using ROC analysis. Cutoff values of concentration for the overexposure to CNI were determined by ROC analysis for ARI, referring to previous reports [29–32]. And the rate of overexposure to CNI was defined as patient proportion with averaged tacrolimus trough >10 ng/ml or with cyclosporine trough >200 ng/ml. The variables examined were age, sex, background disease, Model for End-stage Liver Disease (MELD) score, pre-existence of insulin-controlled diabetes mellitus and hypertension at transplantation, donor age, graft and graft volume,

