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ORIGINAL ARTICLE

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Hepatocyte growth factor and transforming growth factor β1 contribute to regeneration of small-for-size liver graft immediately after transplantation

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Abstract Although the ability of the liver to regenerate to a predetermined size after resection made adult-to-adult living donor liver transplantation (LDLT) possible, there is little information regarding the growth regulatory mechanism for a small-for-size graft. Forty-one cases of LDLT were divided into two groups by graft volume to standard liver volume ratio (GV/SLV); small graft group (Group S, GV/SLV < 40%, $n = 16$) and non-small graft group (Group NS, GV/SLV > 40%, $n = 25$). The regeneration rate (GV at 1 week/harvested GV) and serum levels of hepatocyte growth factor (HGF), transforming growth factor-α (TGF-α) and transforming growth factor-β1 (TGF-β1) were compared between two groups. The regeneration rates in Group S were significantly higher than that of Group NS (217 ± 12% and 178 ± 10%, respectively, $P < 0.01$). The serum HGF levels of Group S were significantly higher than those of Group NS on POD 1. The TGF-β1 levels of Group S were significantly higher than those of Group NS on POD 3 and 5. The TGF-α levels were not different at any time points studied. These results indicate that a small-for-size graft retains the capacity to regenerate faster by modulation of expression pattern of HGF and TGF-β1 immediately after LDLT. After the acceleration of the regenerative response by HGF, subsequent elevation of TGF-β1 synergistically controls graft size, regulating uncontrolled proliferation of hepatocytes.

Keywords Liver regeneration · Small grafts · CT volumetry · Regeneration rates

Introduction

Liver transplantation from living donors is being increasingly performed and accepted as a valuable treatment for patients with end-stage liver disease [1, 2, 3]. One problem with adult-to-adult living donor liver transplantation (LDLT) is reduced parenchymal mass for adequate function in the recipient [4, 5]. Fortunately, the liver has the capacity to regulate its growth and size in response to the demands of the body on hepatic function. The grafted liver, which is of a small size in relation to a particular recipient, will grow in the recipient until it reaches its optimal mass, at which point the growth of the organ ceases. In contrast, the grafted liver of larger than optimal size for a recipient will not grow, and might even decrease in mass [6].

Many growth regulatory factors for hepatocytes have been found since hepatocytes in primary culture were utilized [7, 8]. Hepatocyte growth factor (HGF), transforming growth factor-α (TGF-α), Epidermal growth factor (EGF) and Interleukin-6 (IL-6) can stimulate hepatocyte proliferation in vivo as well as in vitro [9, 10, 11, 12, 13, 14]. In contrast, transforming growth factor-β1 (TGF-β1) is reported to inhibit hepatocyte proliferation [15]. Although it is thought that these growth regulatory factors interact and synergistically modulate

hepatic regeneration to converge to the standard liver volume, little is known about how these factors promote small-for-size liver graft to regenerate. Understanding the mechanism by which the transplanted liver undergoes regeneration is clinically important and may help to devise ways to accelerate liver regeneration in patients receiving small grafts.

To obtain some insight into these questions, we investigated a serial change of serum HGF, TGF-α, TGF-β1 and regeneration rate of grafted liver using CT volumetry immediately after LDLT and compared these parameters between small graft group (Graft Volume/Standard Liver Volume < 40%) in which liver growth occurs rapidly and non-small graft group (GV/SLV > 40%) in which liver growth occurs gradually.

Patients and methods

Patients

From October 1996 to August 2001, 59 LDLTs for 57 patients were performed at the Kyushu University Hospital with the approval of the Kyushu University Ethics Committee. Among them, 41 patients, whose blood samples were available, were included in this study. The patients were 14 males and 27 females, between 7 and 65 (mean ± SEM: 41 ± 3) years. Data on the donors and recipients, classified by graft volume as defined later, are shown in Table 1. The recipient's standard liver volume (SLV) was calculated according to the formula by Urata et al. [16]: SLV (ml) = 706.2×body surface area (m^2) + 2.4. The weight of the harvested liver segment, assigned as the GV, was measured on the back table. According to the GV divided by SLV (GV/SLV), the 41 patients were divided into two groups: non-small graft group (Group NS), GV/SLV > 40% (n = 25) and small graft group (Group S), GV/SLVn = 16).

Measurement of serum HGF, TGF-α and TGF-β1

Blood samples were obtained 2 h after reperfusion and on days 1, 3, 5, 7 after transplantation. Samples were collected into a serum separator tube and centrifuged for 10 min at 1000 g. Sera were stored at −80°C until assay was performed. The serum TGF-β1 concentrations were measured with a TGF-β1 ELISA kit according to the manufacturer's instructions (R&D Systems, Minneapolis) which detects biologically active TGF-β1 as total TGF-β1. Prior to assay, serum samples were acid-activated and neutralized to activate latent TGF-β1 to the immunoreactive form [17]. The serum HGF levels were also determined in the original serum with a HGF ELISA kit (Otsuka Pharmaceutical, Tokyo, Japan). The serum TGF-α levels were determined with a TGF-α ELISA kit (Oncogene Research Products, San Diego, Calif.).

Table 1 Demographic variables. [a] GV/SLV, graft volume/standard liver volume. [b] GRWR, graft-to-recipient weight ratio. [c] RBC, red blood cell

	Group S (n = 16)	Group NS (n = 25)	
Donors			
Age (years)	35.2 ± 3.1 (21–65)	41.2 ± 2.2 (21–60)	N.S.
Gender (Male/Female)	8/8	18/7	N.S.
Operation time (min)	460 ± 26	445 ± 15	N.S.
Blood loss (ml)	869 ± 110	1123 ± 179	N.S.
Graft			
Extended lateral segment	0	1	
Extended left lobe	6	7	
Extended left lobe + caudate	10	11	
Right lobe	0	6	
Graft Volume (g)	344 ± 17 (230–460)	526 ± 21 (370–760)	$P < 0.001$
GV/SLV [a] (%)	32.2 ± 1.2 (23–39)	51.8 ± 2.5 (41–86)	$P < 0.001$
GRWR [b] (%)	0.67 ± 0.04 (0.41–0.95)	1.14 ± 0.09 (0.74–2.54)	$P < 0.001$
Cold ischemia time (min)	67.1 ± 9.4	74.6 ± 8.3	N.S.
Rewarming time (min)	39.2 ± 1.4	44.2 ± 2.6	N.S.
Recipient			
Age (years)	42.8 ± 4.4 (13–65)	40.0 ± 3.4 (7–63)	N.S.
Gender (Male/Female)	8/8	6/19	N.S.
Adult/Child	14/2	21/4	
Operation time (min)	812 ± 66	819 ± 49	N.S.
Blood loss (ml)	7278 ± 1327	6926 ± 1247	N.S.
RBC [c] transfusion (Units)	25.6 ± 6.2	21.8 ± 4.3	N.S.
Indication for LDLT			
Fulminant hepatic failure	5	5	
Metabolic liver diseases	0	3	
Liver cirrhosis	2	4	
Primary biliary cirrhosis	4	8	
Biliary atresia	2	5	
Primary sclerosing cholangitis	1	0	
Others	2	0	
Liver function tests at 7POD			
Total Bilirubin level (mg/dl)	7.7 ± 1.5	6.5 ± 1.0	N.S.
ALT level (IU/l)	175 ± 30	174 ± 31	N.S.
Prothrombin time (second)	14.9 ± 0.6	14.4 ± 0.6	N.S.

Determination of regeneration rate

Computed tomography (CT) 1 week after transplantation was used for graft volumetric analysis [18]. Regeneration rate at 1 week after transplantation was calculated as graft volume 1 week after LDLT divided by harvested graft volume at operation. Since the edematous change of liver parenchyma may play a role in analyzing volume by CT, the regeneration rate was also assessed applying the graft-to-recipient weight ratio (GRWR, percentage of graft volume in the recipient body weight). The regeneration rate was calculated as GRWR at 1 week divided by GRWR at operation.

Statistical analysis

Data were expressed as mean ± SEM. Statistical analysis was performed using Student's t test. Differences at $P < 0.05$ were considered significant.

Results

Operative data

The operative time and blood loss (Table 1) during the donor and recipient surgery did not differ between the groups. The harvested graft weights were 344 ± 17 g in Group S and 526 ± 21 g in Group NS ($P < 0.001$). The GV/SLV ratio were 32.2 ± 1.2% in Group S and 51.8 ± 2.5% in Group NS ($P < 0.001$). Cold ischemic time in all the transplant procedures was within 2.5 h. There were no significant differences in postoperative complications of recipients between the groups.

Post-transplant graft function

There were no significant differences in postoperative serum ALT or bilirubin level between the groups, nor were there any significant differences in postoperative prothrombin time (Table 1).

Graft liver regeneration

The regenerated graft volumes determined by CT volumetric analysis at 1 week after transplantion were 751 ± 27 ml in Group S and 830 ± 17 ml in Group NS. There were no statistically significant differences between two groups in terms of regenerated graft volume at 1 week. The regeneration rates of the graft are shown in Fig. 1. The regeneration rates, defined as post-transplant 1 week GV/harvested GV, in Group S were significantly higher than those of Group NS (217 ± 12% and 178 ± 10%, respectively, $P < 0.05$). To confirm that these results were not affected by the liver parenchymal edema, the regeneration rates were also evaluated using GRWR. The regeneration rates defined by the change of GRWR were 233 ± 15% in Group S and 178 ± 10% in group NS, and the values were significantly different ($P < 0.01$).

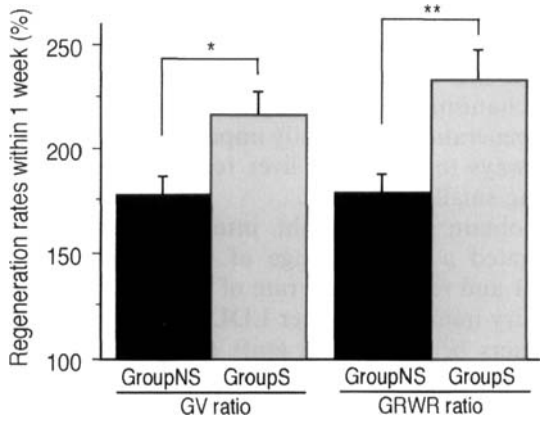

Fig. 1 The regeneration rate of the graft was determined using two parameters. Graft Volume (*GV*) ratio, defined as post-transplant 1 week GV / harvested GV. Graft-to-recipient weight ratio (*GRWR*) ratio, defined as GRWR at 1 week / GRWR at operation. *Group S* had significantly greater values of regeneration rate than that of Group NS. Values are expressed as mean ± SEM. $*P < 0.05$. $**P < 0.01$. Statistical significance was determined by Student's t test

Post-transplant serum HGF, TGF-α and TGF-β1 levels

Figure 2 shows the serial changes in HGF concentrations of both groups. It is known that serum levels of HGF in patients with fulminant hepatic failure (FHF) were increased in relation to hepatocellular dysfunction and necrosis [19, 20]. In this study, as shown in Table 1, 5 cases of FHF were included in each group. In accordance with previous reports, preoperative serum HGF levels in FHF cases ($n = 10$) were extremely high (32.7 ± 5.0 ng/ml), and preoperative levels in all cases (Group S, 10.1 ± 4.0 ng/ml; Group NS, 7.6 ± 3.2 ng/ml, respectively; $P = 0.63$) were abnormally elevated as compared with post-operative data. Therefore, preoperative values were omitted in Fig. 2A. It is thought that preoperative serum HGF values have little influence on post-operative values because, as previously reported, HGF is cleared by liver, adrenal, spleen and kidney, and its half-life is estimated to be less than 10 min [21, 22]. As shown in Fig. 2A, serum HGF levels of Group S were significantly higher than those of Group NS on POD 1 (1.6 ± 0.3 ng/ml and 0.9 ± 0.2 ng/ml, respectively, $P < 0.05$). To confirm that the differences in HGF levels observed on POD 1 were not modified by pre-existed high HGF levels due to FHF cases, the data of patients without FHF cases were shown in Fig. 2B. The statistical significance was also observed on POD 1 (Group S, 1.7 ± 0.3 ng/ml; Group NS, 0.8 ± 0.2 ng/ml, $P < 0.05$).

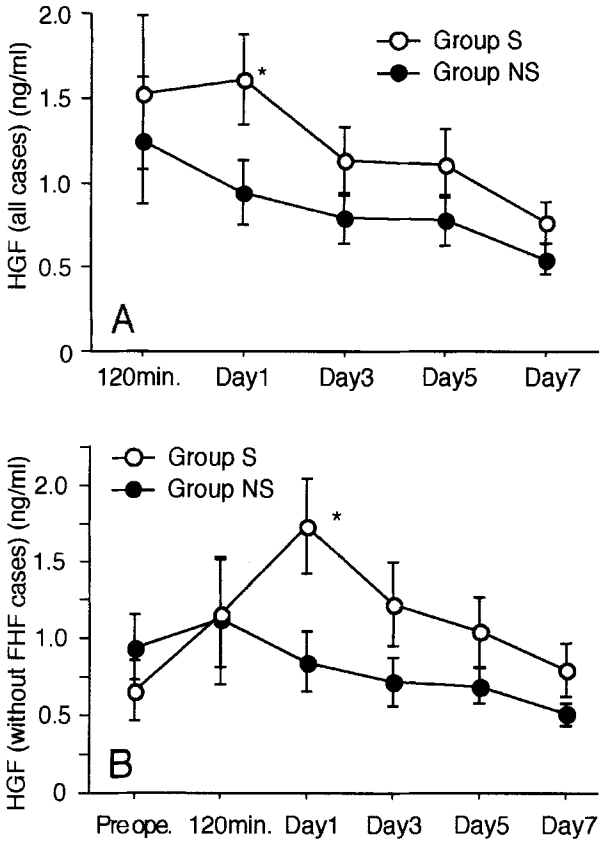

Fig. 2 Change in serum HGF levels of all cases **A** and without fulminant hepatic failure (*FHF*) cases **B** are shown. **A** *Group S* had significantly higher values of serum HGF than those of *Group NS* on POD 1. **B** Almost the same results were observed, even when FHF cases were excluded. Values are expressed as mean ± SEM. $*P < 0.05$ vs. Group NS at the same time point. Statistical significance was determined by Student's *t* test

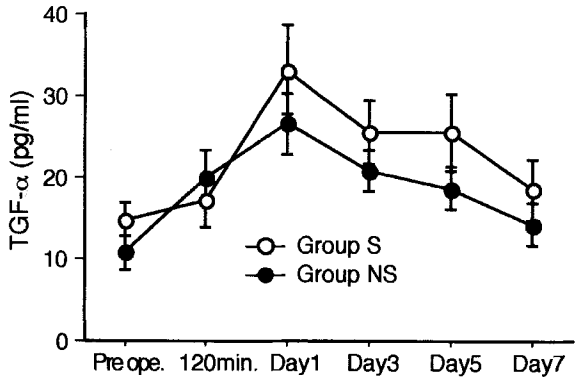

Fig. 3 Change in serum TGF-α levels by graft size are shown. Statistical significance was not observed between the groups at any time points studied. Values are expressed as mean ± SEM. $*P < 0.05$ vs. Group NS at the same time point. Statistical significance was determined by Student's *t* test

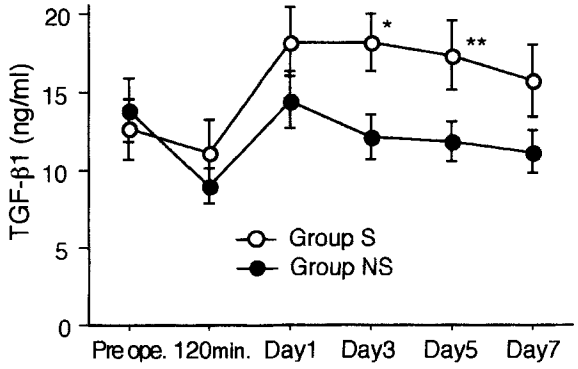

Fig. 4 Change in serum TGF-β1 levels by graft size are shown. *Group S* had significantly higher values of serum TGF-β1 on POD 3 and 5. Values are expressed as mean ± SEM. $*P < 0.01$ vs *Group NS* at the same time point. $**P < 0.05$ vs. Group NS at the same time point. Statistical significance was determined by Student's *t* test

As shown in Fig. 3, serum TGF-α rose after operation, and it reached a maximum level at POD 1. However, statistical significance was not observed between the groups at any time points studied.

Figure 4 shows the serial changes in TGF-β1 concentrations of both groups. Serum levels of TGF-β1 were slightly decreased 2 h after reperfusion, and elevated thereafter. Comparing the two groups, the values of Group S seemed to be higher than those of Group NS after POD 1, and statistical significance was observed on POD 3 (19.7 ± 2.5 ng/ml and 11.5 ± 1.3 ng/ml, respectively, $P < 0.01$) and POD 5 (19.7 ± 2.9 ng/ml and 12.0 ± 1.4 ng/ml, respectively, $P < 0.05$).

Discussion

The unique ability of the liver to regenerate to a predetermined size after resection or transplantation makes adult-to-adult living donor liver transplantation possible. The adult hepatocyte is normally a quiescent, highly differentiated cells. However, almost immediately, it can begin the process of replication in response to conditions that induce cell loss due to physical or toxic injury. Furthermore, the events involved in the regenerative response are precise, carefully orchestrated, and highly regulated in order to respond to functional deficiencies as efficiently as it does to excess capacities. For example, transplanted livers grow in size until optimal mass is achieved; in contrast, those that are too large for the recipient decrease in size, probably by undergoing apoptosis. Many growth factors, most notably HGF and TGF-α are reported to stimulate hepatocyte proliferation. However, little has been reported on a growth regulatory mechanism of small-for-size liver graft in LDLT.

In our study, the liver grafts of the small graft group (GV/SLV < 40%) recovered their volumes as well as the non-small graft group at 1 week, and the regeneration rates were higher in the small graft group. From these observations, it could be inferred that differential modulation of growth regulatory mechanism existed between the two groups. As expected, serum HGF levels of the small graft group were significantly higher than those of the non-small graft group at POD 1. After 70% hepatectomy in rodent model, plasma concentrations of HGF rise more than 17-fold within 2 h and remain elevated for the first 72 h [23]. Subsequent peak of DNA synthesis in remnant livers occurred at 24 h after hepatectomy.

Surprisingly, the small graft group maintained significantly higher values of serum TGF-β1, which is well known to be one of the most potent growth inhibitor of hepatocytes, than the non-small graft group at POD 3 and 5, and tended to be higher throughout the observation period. It may seem to be paradoxical that there were higher levels of growth inhibitory signals in the small graft group in which faster regenerative response has occurred. Braun et al. showed that TGF-β1 mRNA markedly increased in the rat liver after 70% hepatectomy and reached a maximum after the major prominent peak of DNA synthesis of hepatocytes [24]. Our findings are compatible with theirs in terms of the regulation of uncontrolled hepatocyte proliferation by TGF-β1. This phenomenon is reasonable considering that, after one-third hepatectomy, restoration of the original number of hepatocytes theoretically requires only 1.66 proliferative cycles per residual hepatocyte. This seemingly paradoxical sequence of events may be an important mechanism that prevents uncontrolled growth during liver regeneration. There may be a balance between stimulators and inhibitors such that the balance favors DNA synthesis induction during the immediate early phase after reperfusion but gradually shifts toward inhibition in subsequent days.

It was also reported that TGF-β1 synergistically enhances growth factor stimulated hepatocyte motility responses during tissue remodeling [25]. This function may be one of the most important factors in the process of liver regeneration. In the early stage of regeneration, hepatocyte clusters without intervening sinusoids are formed as a consequence of hepatocyte division. Subsequent reestablishment of normal vascular architecture by remodeling is thought to begin at 96 h after regenerative response had initiated, corresponding to the time that elevated TGF-β1 levels were observed in this study [8]. Typical hepatic lobular architecture is gradually restored through remodeling steps.

In conclusion, our present study revealed that the small-for-size graft retains the capacity to regenerate faster by modulation of expression pattern of HGF and TGF-β1 immediately after LDLT. Namely, HGF accelerates hepatic regeneration immediately after operation,and subsequent elevation of TGF-β1 might have played a role to synergistically control the graft size to converge to the standard liver volume.

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