less than 32 and a triple immunosuppressive regimen including tacrolimus, steroids and MMF after LDLT (17). MMF was started 7 days before LDLT. In the LL cohort, only eight patients (4%) had ABO incompatible grafts, whereas 154 had identical and 38 had compatible grafts (Table 1).

Definition of grade of donor postoperative complications

The postoperative complications of the donor were graded according to the modified Clavien classification (18). A postoperative peak total bilirubin level >5mg/mL was defined as Clavien grade 2.

Definition of small-for-size syndrome

The definition of SFSS was as reported previously (5,6). Briefly, SFSS is defined as having prolonged functional cholestasis (total bilirubin >10 mg/dL at postoperative day 14) and intractable ascites (daily production of ascites of >1 L at postoperative day 14 or >500 mL at postoperative day 28).

Statistical analysis

Continuous variables were compared using a two-tailed, unpaired Student t-test for independent samples. All values are expressed as mean ±standard deviation. Categorical data were compared using the chi-square test. Analysis of patient survival was performed using the Kaplan–Meier method and compared between groups using the log-rank test. p-Values <0.05 were considered significant. All statistical analyses were done using SPSS 17.0 (SPSS Inc., Chicago, IL).

Results

Patient characteristics

There were no significant differences in patient age and MELD score between RL and LL groups (Table 1). The mean GV of LL grafts was 432 g (range 220–750 g), which was significantly smaller than that of RL grafts (566 g, range 395–760g, $p < 0.0001$). The mean GV/SLV ratio and GRWR were 38.7% (range, 21.0–66.1%) and 0.82% (range, 0.41–1.51%), respectively in LL grafts, which were, again, significantly smaller than those of RL grafts (47.4% and 0.9%, respectively). Twenty-one LL grafts were extremely small, namely, GV/SLV <30%, although the preoperative predicted GV/SLV was >35% in 17 grafts. The smallest LL graft GV/SLV was 21.0%, for which auxiliary partial orthotopic liver transplantation was performed in patients with primary sclerosing cholangitis (8). Hepatocellular carcinoma was the main indication both in LL (42.5%) and RL (44.6%) LDLT.

Overall patient and graft survival rates

As shown in Figure 2A, the cumulative overall 1-, 5- and 10-year patient survival rates were 85.6%, 77.9% and 69.5%, respectively, in patients with LL grafts, which were comparable to those of patients with RL grafts. The cumulative 1-, 5- and 10-year graft survival rates were similar, 84.0%, 76.5% and 59.6%, respectively, in LL grafts, which again were comparable to those of RL grafts (Figure 2B). Figure 3 shows patient survival in LL grafts according to the GV/SLV ratio. To investigate the impact of the graft size, the GV/SLV ratio was classified into four subgroups as follows: <30% (n = 21); ≥30%, <35% (n = 43); ≥35%, <40% (n = 51) and ≥40% (n = 85). There were no significant differences in patient and graft survival rates between these subgroups (Figure 3). Furthermore, 119 (59.5%) out of 200 LL grafts in our series were GRWR <0.8. The 1- and 5-year graft survival rates of this group of patients were 84.1% and 75.6%, respectively, which were comparable to those of patients with LL grafts of GRWR ≥0.8 (83.7% and 76.3%).

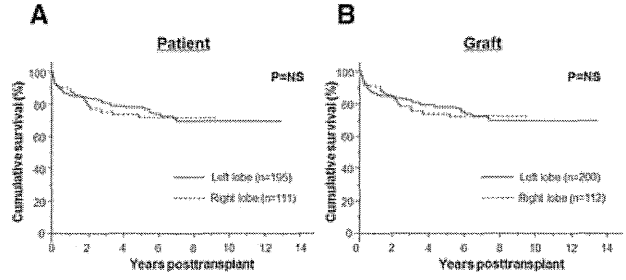

Figure 2: Comparison of cumulative patient (A) and graft (B) survival rates between left lobe (LL) and right lobe (RL) living donor liver transplant (LDLT).

Donor operative outcomes

Table 2 shows the comparison of operative outcomes between LL and RL donors. The mean operative time was comparable whereas blood loss was significantly less in RL donors (493 mL vs. 649 mL). However, we did not give homologous blood transfusion to any of the LL and RL donors. Postoperative liver function tests including peak total bilirubin, peak aspartate aminotransferase and alanine aminotransferase were significantly better in LL donors. Furthermore, lengths of hospital stay were significantly shorter in LL donors (12.2 days vs. 17.3 days), whereas overall morbidity rates were comparable. These data suggest that LL donation is potentially safer than RL donation, although there was no procedure-related mortality in either group. Ten LL donors with gastric stasis (n = 5), biloma/bile

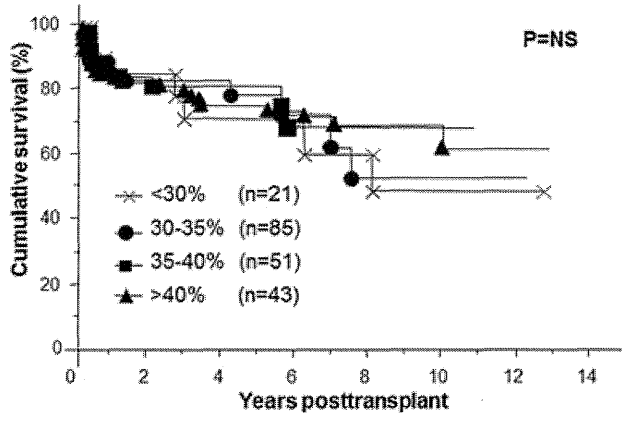

Figure 3: Comparison of cumulative graft survival according to GV/SLV ratio in LL LDLT. The log-rank test found no statistically significant differences.

Table 2: Donor outcomes

Factors	Left lobe (n = 200)	Right lobe (n = 112)	p-Value
Donor			
Operative time (min)	448 ± 78*	449 ± 71	NS
Blood loss (mL)	659 ±5 01	493 ± 327	0.0025
Blood transfusion (%)	0	0	NS
Postoperative LFTs			
Peak T.Bil (mg/dL)	2.4 ± 1.4	3.1 ± 1.5	<0.0001
Peak AST (IU/L)	482±254	562±279	0.013
Peak ALT (IU/L)	529 ± 265	604 ± 351	0.038
Morbidity (%)	36.0	34.8	NS
Clavien I	14.5	13.4	
Clavien II	14.0	11.6	
Clavien IIIa	5.0	7.1	
Clavien IIIb	2.5	2.7	
Clavien IV	0	0	
Clavien V	0	0	
Hospital stay (days)	12.2 ± 5.2	17.3 ± 10.0	<0.0001

*Mean ±SD. T.Bil = total bilirubin; AST = aspartate amino transferase; ALT = alanine amino transferase.

leakage (n = 3) and wound sequelae (n = 2) and eight RL donors with biloma/bile leakage (n = 2), bile duct strictures (n = 2) and pneumothorax/pleural effusion (n = 4) had non-surgical intervention (Clavien's IIIa). Furthermore, five LL donors (2.5%) with incisional hernia (n = 2), bile leakage from the closed stump of the left hepatic duct (n = 1), bile duct strictures (n = 1) and postoperative bleeding (n = 1) and three RL donors (2.7%) with bile duct stricture (n = 1), an incisional hernia (n = 1) and a cosmetic wound defect (n = 1) underwent reoperation (Clavien's IIIb). Two donors (19-year-old male LL and 56-year-old male RL donors) with normal liver function tests died from suicide and an unknown sudden cardiovascular cause 5 years and 1 year, respectively after donation. A 47-year-old male donor developed chronic myeloid leukemia 4 years after donation for whom imatinib was given to achieve complete remission. In terms of procedure-related complications, we have not experienced any Clavien's grade IV and V complications so far.

Recipient operative outcomes

Table 3 shows a comparison of operative data between LL and RL recipients. The mean operative time was approximately 2 h longer in RL recipients and RL recipients more often required V-V bypass. Concomitant splenectomy was performed in 36% of LL and 47.3% of RL recipients. A temporary PCS was created during the anhepatic phase in 16.5% of LL recipients because of SFS grafts with GV/SLV <35% (n = 7), fulminant hepatic failure (n = 9) and an absence of liver cirrhosis (n = 2) and other reasons (n = 12), compared with 9.0% of RL recipients. The mean GV/SLV of patients who had a temporary PCS was 36.9%.

Figure 4 compares the 1-year graft survival rates between LL and RL LDLT according to the MELD scores. In all categories, the LL group revealed comparable results with the RL group. However, in patients with a MELD score >30, the LL group (n = 8) tended to show the worst outcome compared with the RL group (n = 7) (1-year graft survival 50% vs. 66.7%). Four LL patients with a MELD score >30 were lost because of hepatic artery thrombosis (n = 1), graft dysfunction and PVT (n = 1), hepatic infarction because of portal infusion therapy (n = 1) and graft dysfunction and subarachnoid hemorrhage (n = 1), whereas only one patient with a RL graft was lost because of hepatocellular carcinoma recurrence. Even though there was no statistical significance, the decrement in outcomes for LL grafts in high MELD patients is obvious. Therefore, RL grafts should be considered first over LL grafts for very sick patients with MELD score >30.

Incidence of small-for-size syndrome

The incidence of SFSS was higher in LL LDLT (19.5%) than in RL LDLT (7.1%) ($p < 0.01$). The mean GRWR in the patients with who developed SFSS was 0.74, which was comparable to those without SFSS (0.78, p = NS). Therefore, graft size is not the sole determinant to develop SFSS in our series.

Cause of graft loss

Of the 200 LL grafts, 54 grafts were lost because of hepatic artery thrombosis (n = 2), chronic rejection (n = 4), hepatic infarction (n = 5), graft dysfunction/sepsis (n = 8), including SFSS (n = 3), graft-versus-host disease (n = 1), recurrent hepatitis C (n = 5), recurrent hepatocellular carcinoma (n = 10), *de novo* malignancy (n = 1) and other causes (n = 18) including hepatic abscess (n = 1), suicide (n = 1), drowning (n = 1), cardiac failure (n = 1), pancreatic fistula leading to rapture of pseudoaneurysm (n = 1), subarachnoid hemorrhage (n = 1), respiratory failure (n = 1), adult T-cell leukemia (n = 1), cholangitis (n = 1), sepsis after biliary stenting (n = 1), procedure-related iatrogenic bleeding (n = 2) and arterio-portal fistula (n = 1), uncontrollable bleeding during transplant (n = 2), late portal vein thrombosis (n = 1), recurrent PBC (n = 1), colonic perforation (n = 1), late-onset acute rejection (n = 1). Among these, 23

Table 3: Recipient outcomes

Factors	Left lobe (n = 200)	Right lobe (n = 112)	p-Value
Operative time (min)	766 ± 151*	893 ± 201	<0.0001
Blood loss (mL)	6929 ± 17 073	7485 ± 7815	NS
Blood transfusion			
PRBC (U)	17.9 ± 29.4	21.9 ± 19.2	NS
FFP (U)	18.5 ± 21.4	26.6 ± 20.1	0.001
PLT (U)	16.8 ± 24.2	23.1 ± 20.4	0.02
Portal pressure (mmHg)			
At laparotomy	21.7 ± 6.2	20.9 ± 6.4	NS
Before closure	16.3 ± 3.5	16.2 ± 4.0	NS
Portal flow (mL/min)	1423 ± 584	1870 ± 693	<0.0001
Portal flow (mL/min/g liver)	3.35 ± 1.40	3.35 ± 1.25	NS
V-V bypass (%)	5.5	36.7	<0.0001
Splenectomy (%)	36.0	47.3	NS
SA ligation (%)	8.0	6.3	NS
Temporal portocaval shunt (%)	16.5	8.9	NS
Permanent hemiportocaval shunt (%)	1.0	0	NS
Complications (%)			
SFSS	19.5	7.1	0.0063
HAT	2.0	1.7	NS
PVT	2.0	0.9	NS
ACR	16.0	17.0	NS
Bile leak	6.5	5.4	NS
Bile duct strictures	20.0	17.0	NS
Relaparotomy	15.0	8.9	NS
In-hospital mortality (%)	12.0	8.0	NS

*Mean ±SD. PRBc = packed red blood cells; FFP = fresh frozen plasma; PLT = platelet; V-V = veno-venous; SA = splenic artery; SFSS = small-for-size graft syndrome; HAT = hepatic artery thrombosis; PVT = portal vein thrombosis; ACR = acute cellular rejection.

(42.6%) were lost within 3 months after LDLT. In particular, hepatic infarction was probably associated with catheterization for portal infusion therapy, which had been used for some time periods. On the other hand, 27 RL grafts were lost because of chronic rejection (n = 4), recurrent hepatitis C (n = 2), recurrent hepatocellular carcinoma (n = 4), multiple liver abscess (n = 1), graft dysfunction/sepsis (n = 7), anterior segment congestion (n = 1) and other causes (n = 8) including adult T-cell leukemia (n = 1), biopsy-related liver hematoma (n = 1), *de novo* autoimmune hepatitis (n = 1), recurrent colon cancer (n = 1), esophageal cancer (n = 1), recurrent epithelioid hemangioendothelioma

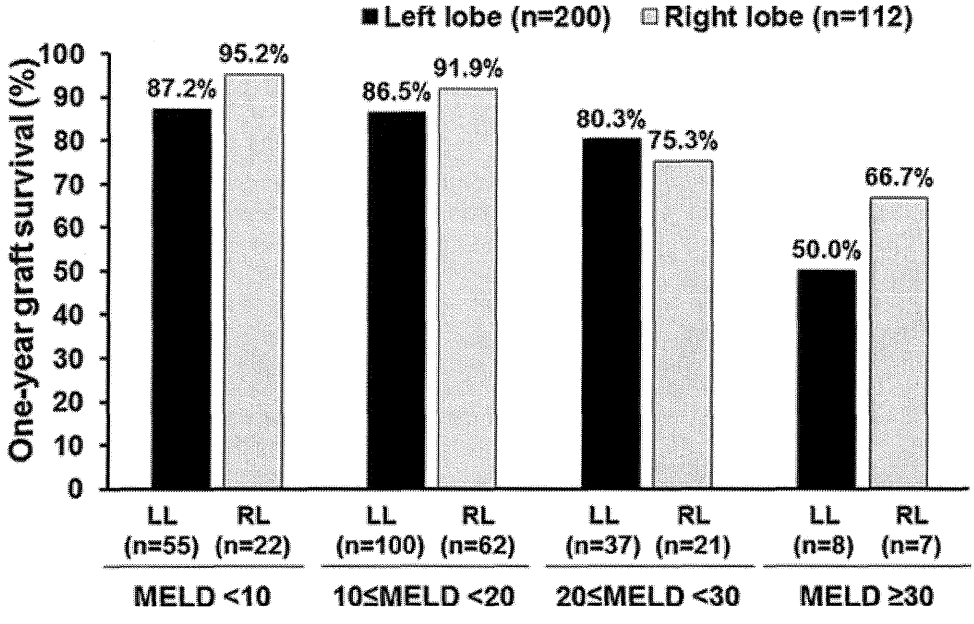

Figure 4: Comparison of LL and RL graft survival rates according to the MELD score. There was no significant difference between the two types of graft at any level of the MELD score.

(n = 1), acute renal failure (n = 1) and nonocclusive mesenteric ischemia (n = 1). Among these, eight cases (29.6%) were lost within 3 months after LDLT.

Discussion

This study clearly showed that the outcomes of LL LDLT were comparable with those of RL LDLT, although SFSS occurred more often in LL LDLT. However, this does not necessarily lead to graft loss. In our cohort, only three patients lost their grafts directly as a result of SFSS.

SFSS is characterized clinically by a combination of prolonged functional cholestasis, intractable ascites and a delayed recovery of both prothrombin time and encephalopathy (19). The mechanism of SFSS remains unknown but is probably multifactorial. Excessive portal perfusion and pressure to the small graft is suggested to be one of the most important factors (20). Therefore, some groups have advocated the use of temporary PCS to reduce or minimize the influence of detrimental substances accumulating during portal clamping (21,22). Troisi et al. reported that an intentional decrease in portal flow by HPCS improved the survival of patients who received LL grafts with a GW/RW <0.8 (23). Yamada et al. selectively used HPCS for LL grafts with GW/RW between 0.6 and 0.8 and showed 100% patient survival (24). Botha et al. also reported excellent results in patients with small LL grafts (the median GW/RW was 0.67) with HPCS: the 1-year patient and graft survival were 87% and 81%, respectively (25). They all concluded that a small LL graft with modulation of portal flow by HPCS may prevent SFSS while at the same time providing adequate liver volume. Furthermore, the Kyoto group showed that portal venous pressure <15 mmHg was the major factor for a better outcome (26). However, we used HPCS in only two patients, one with a very small-for-size graft (GV/SLV of 24%) whose HPCS was closed 4 days after LDLT because of the portal steal phenomenon and one with a small graft (GV/SLV of 27%) with excessive portal flow. Therefore, we do not think HPCS is always necessary to prevent SFSS.

Our current approach in managing the problem of SFSS is to perform splenectomy aggressively. In the last 50 LL cases, splenectomy was performed for 35 cases (70%) whereas seven cases (14%) had already had splenectomy before LDLT. We have had only three cases (6%) with SFSS out of the last 50 cases and two of the three cases recovered from the complication whereas the other required retransplantation. In terms of the usefulness of splenectomy for low GRWR (<0.8) patients, the 1-year graft survival rates in patients with splenectomy were 93.4%, which was significantly better than those without splenectomy (79.2%) (data not shown). Therefore, we believe concomitant splenectomy is very useful especially for patients with a small graft to control the portal flow and platelet count, thereby improving the overall results.

We did not set strict definition of the "excessive" portal flow or portal pressure. However, we think that portal flow more than 2500 mL/min or 500 mL/min per 100 g liver and portal pressure more than 20 mmHg are both detrimental to the graft. Therefore, Portal flow modulation should aggressively be tried for these patients. In a successful case with extremely small graft (GV/SLV 23%), the portal flow to the LL graft was reduced to 270 mL/min with the combination of a permanent hemi PCS and splenectomy. In another case with an extremely small graft (GV/SLV 27.2%), the portal flow before hemi PCS was 2500 mL/min, which was decreased to 1000 mL/min with a hemi PCS and splenectomy. Both of the cases were successful without small-for-size syndrome.

Technically speaking, LL LDLT is simpler than RL LDLT as indicated by the shorter length of the operative time. LL grafts usually have a single hepatic vein, a single portal vein and a single bile duct although hepatic artery reconstructions are sometimes multiple. On the other hand, RL LDLT requires additional reconstructions of MHV tributaries and multiple bile duct reconstructions, which prolongs the total operative time.

In terms of donor safety, our data confirmed the results of published series (27,28), which revealed the superiority of LL donation over RL donation. Gastric stasis, which is a specific complication after LL donation, occurred in 12 cases in LL donors (6%). Among these, five required endoscopic correction. This complication probably results from rotation of the distal stomach and the duodenum adhering to the raw surface of the remnant liver, therefore this could be prevented by using an antiadhesive film such as hyaluronic acid-carboxymethylcellulose membrane (Seprafilm®; Genzyme Corp., Cambridge, MA, USA) before closing the abdomen or by early resumption of feeding after donation. The film should be attached to the surface of the antropyloric region of the stomach (not to the cut surface of the liver) just before closing the abdomen.

There is a discussion that LL LDLT should not be used in large patients, especially those in Western countries. We believe this is not necessarily true provided that the LL donor is as large as the recipient. In fact, our data showed that LL donors were more often male and LL LDLTs were more often given to smaller female recipients. On the other hand, RL donors were more often female whereas RL recipients were more often male. In our 200 LL cohort, 5 patients were heavier than 80 kg whereas 22 patients were more than 70 kg. There was no patient heavier than 90 kg. Among them, only a female patient of 81 kg body weight (GV/SLV 39.7% and GRWR 0.6) died early (<3 months after LDLT) because of SFSS and sepsis. The other 20 patients (95%) survived LDLT. Therefore, we insist that as far as the donor LL grafts have sufficient GV (GV/SLV > 35% or GRWR > 0.8), LL LDLT should be feasible even for heavier Western patients.

Table 4: Left lobe living donor liver transplantation in adults: world experience

Ref. #	Author	Year	Patients (n)	GV/SLV* (%)	GRWR* (%)	Portal flow modulation (%)				SFSS** (%)	Survival (%)	
						NO	HPCS	SPL	SAL		1-Year	5-Year
29	Kawasaki	1998	13	40.2	NA	100	0	0	0	NA	NA	NA
30	Miller	2001	9	NA	0.69	100	0	0	0	44.0	78.0	NA
7	Soejima	2006	107	40.5	0.81	78.5	0.0	7.5	15.0	25.2	81.4	74.7
24	Yamada	2008	7	NA	0.65	14.3	85.7	0	0	0	NA	NA
31	Ikegami	2009	120	39.9	NA	100	0	0	0	0.8	87.5	80.1
25	Botha	2010	21	NA	0.67	23.8	76.2	0	0	4.8	87.0	NA
32	Ishizaki	2011	42	39.8	NA	100	0	0	0	0	100.0	91
Present series	Soejima	2011	200	38.7	0.82	43.5	1.0	36.0	8.0	19.5	85.6	77.9

*Mean, **definition of SFSS differ between the studies. NA = not available; GV/SLV = graft-to-standard volume ratio; GRWR = graft-to-recipient weight ratio; HPCS = hemiportocaval shunt; SPL = splenectomy; SAL = splenic artery ligation; SFSS = small-for-size syndrome.

With refinement of surgical procedures, postoperative management as well as better graft and patient selection, we have achieved significant progress in cases and outcomes. I summarized the world experience of LL LDLT in Table 4 (29–32). The 1-year patient and graft survival rates in the last 50 LL LDLT in our series is now >95% (data not shown). We therefore currently think that adult LDLT can be successful with either a LL or a RL graft provided that an appropriate graft is selected.

Finally, we summarize our current criteria and recommendation for adult LDLT. The LL grafts is not only a viable option for adults patients but the procedure that should be considered first except for a sick patient with MELD ≥30. The estimated GV/SLV is ideally more than 35% for patients with a MELD score <30 whereas a graft with estimated GV/SLV <35% is still an option for patients with low MELD score without severe portal hypertension. Splenectomy is a viable option to reduce portal flow and pressure especially for patients with a SFS graft. Moreover, the combination of splenectomy and HPCS can be an effective modality for an extra-small graft.

In conclusion, further utilization of LL grafts should be recommended to minimize donor morbidity and mortality while maintaining the outcome for recipients equivalent to that of RL LDLT.

Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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Risk Factors That Increase Mortality After Living Donor Liver Transplantation

Tomoharu Yoshizumi,[1,2,3] Ken Shirabe,[2] Akinobu Taketomi,[2] Hideaki Uchiyama,[2] Noboru Harada,[2] Hideki Ijichi,[2] Masanori Yoshimatsu,[2] Toru Ikegami,[2] Yuji Soejima,[2] and Yoshihiko Maehara[2]

Background. Female liver to male recipient is a well-accepted risk factor for graft loss in cadaveric liver transplantation. However, gender matching is infeasible because of an insufficient number of available donors. No studies have been performed on the role of gender in the field of living donor liver transplantation. This report investigates the effect of gender mismatch on the outcome of living donor liver transplantation.

Methods. A total of 335 patients and donors were classified into four groups according to the following gender combinations: male donor to male recipient group (n=104), male donor to female recipient group (n=120), female donor to male recipient (FM) group (n=59), and female donor to female recipient group (n=52). Patient and graft survival were compared among the groups. We performed a multivariable analysis to identify the factors associated with patient mortality.

Results. 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. The FM group showed significantly shorter patient survival compared with the other three groups. Independent risk factors for patient mortality were: FM group (P=0.006), pretransplant diabetes mellitus (P=0.001), and a model for end-stage liver disease score more than or equal to 20 (P=0.004).

Conclusions. Male recipients of transplants from female donors, pretransplant diabetes mellitus, and a model for end-stage liver disease score more than or equal to 20 have poor survival rates.

Keywords: Donor, Gender, Transplantation, Mismatch.

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The role of gender in the transplantation of body parts such as the kidney, lung, bone marrow, and heart has been extensively studied (*1–4*). In general, today's solid organ donors cannot be matched by gender because of a disparity between supply and demand (*5*). Some reports indicate that gender mismatch has an impact on graft failure, specifically in male recipients of female livers in cadaveric liver transplantation (LT) (*6–8*). Marsman et al. reported that female recipients had a higher incidence of early rejection within 6 months of LT compared with male recipients. They also found decreased graft survival rate in male recipients of female livers (*9*). In contrast, Lehner et al. (*10*) recently reported no significant differences in patient survival in gender-mismatched LT in a single-center database of 1355 recipients.

Donor age, high model for end-stage liver disease (MELD) score, graft size, and portal hypertension are risk factors for graft failure after living donor liver transplantation (LDLT) for patients with chronic liver failure (*11*). Standard liver volume is proportional to body surface area (*12*). Therefore, the difference in body size between males and females sometimes results in a small-for-size graft (SFSG) in males who receive livers from female donors. Data show poor LDLT outcomes with a graft-weight to recipient-weight ratio of less than 0.8 (*13*). Despite this difference between cadaveric LT and LDLT, there are no studies on gender and LDLT. Therefore, the aim of this study was to clarify the effect of gender mismatch on LDLT outcomes.

RESULTS

Table 1 shows a comparison of variables among the four groups classified by gender combination. The distribution of the primary diagnosis was markedly skewed because of the presence of diseases such as hepatitis C (HCV), primary biliary cirrhosis, and primary sclerosing cholangitis (PSC). Operation time and blood loss were greater in the female donor to male recipient (FM) group than in the other three groups. More left lobe was used in the male donor to male

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[1] Department of Surgery and Multidisciplinary Treatment, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.
[2] Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.
[3] Address correspondence to: Tomoharu Yoshizumi, M.D., Department of Surgery and Multidisciplinary Treatment, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Fukuoka 812-8582, Japan.
E-mail: yosizumi@surg2.med.kyushu-u.ac.jp

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TABLE 1. Comparison of variables between the groups classified by gender combination

Variables	MM group (n=104)	FF group (n=52)	MF group (n=120)	FM group (n=59)	*P*
Recipient variables					
Age, yr (range)	52.5 (19–69)	49.4 (18–71)	52.7 (18–73)	49.9 (23–68)	NS
Primary diagnosis					<0.001
Liver cirrhosis					
Hepatitis C (HCC)	61 (49)	18 (15)	41 (33)	27 (17)	
Hepatitis B (HCC)	12 (10)	1 (1)	9 (7)	6 (5)	
Non-B non-C (HCC)	5 (2)	3 (1)	3 (2)	6 (2)	
Alcohol (HCC)	6 (4)	2 (0)	1 (0)	3 (2)	
Fulminant hepatic failure (FHF)	11	7	25	7	
Primary biliary cirrhosis	3	10	31	1	
Primary sclerosing cholangitis	4	1	1	5	
Biliary atresia	1	3	1	1	
Others	1	7	8	3	
Body mass index (kg/m^2)	23.6±3.0	22.4±3.7	23.0±3.6	23.9±3.7	0.08
MELD score	14.0±7.0	15.4±8.5	15.5±8.4	15.6±8.2	NS
Pretransplant DM (yes/no)	24/80	0/52	15/105	12/47	0.001
Operation time (min)	834±174	765±136	751±139	866±209	<0.001
Blood loss (mL)	6498±6940	4936±5296	4617±5006	8676±7884	0.001
Donor/graft variables					
Graft (left/right/posterior)	72/31/1	28/24/0	95/22/3	15/43/1	<0.001
GW-SLW ratio (%)	40.9±8.2	40.9±9.7	44.2±9.0	40.7±7.1	0.01
GW-BW ratio (%)	0.77±0.16	0.82±0.03	0.88±0.02	0.76±0.02	<0.001
ABO (identical/compatible/incompatible)	84/16/3	39/11/2	82/27/10	39/18/2	NS
Consanguinity (yes/no)	100/4	50/2	97/23	33/26	<0.001
Age, yr (range)	31.7 (20–62)	35.9 (20–58)	36.9 (20–65)	40.2 (22–60)	<0.001
Body mass index (kg/m^2)	22.6±3.1	21.4±2.8	22.9±2.6	21.3±2.3	0.001
Operation time (min)	458±80	426±58	443±67	437±76	0.07
Cold ischemic time (min)	86±59	87±61	66±35	122±69	<0.001
Warm ischemic time (min)	41±12	39±13	37±9	45±11	0.001
Blood loss (mL)	582±340	470±288	630±505	531±442	NS

DM, diabetes mellitus; GW, graft weight; SLW, standard liver weight calculated by 706.2×body surface area+2.4; MM, male donor to male recipient; FF, female donor to female recipient; MF, male donor to female recipient; FM, female donor to male recipient; NS, not significant; HCC, hepatocellular carcinoma; FHF, fulminant hepatic failure; MELD, model for end-stage liver disease; BW, body weight.

recipient (MM) and male donor to female recipient (MF) groups compared with the other two groups. Nevertheless, the ratio of graft weight (GW) to standard liver weight (SLW) and the ratio of GW to body weight were higher in the MF group than in the other three groups. LDLT between spouses was included in the FM and MF groups, reducing consanguinity between donors and recipients. Donors in the MM group were younger than those in the other three groups. Cold and warm ischemic times were longest in the FM LDLT group. Most such cases involve the use of a right lobe graft, which requires hepatic vein reconstruction.

In our series, 80 patients died after LDLT. Hepatocellular carcinoma (HCC) recurrence occurred in 14 patients, sepsis in 13 patients, HCV recurrence in 9 patients, graft failure in 9 patients, bleeding in 8 patients, de novo malignancy in 7 patients, chronic rejection in 4 patients, multiple organ failure in 4 patients, fungal infection in 3 patients, and other conditions in 9 patients. There was no difference in cause of death among the groups. Furthermore, 23 patients died in the FM group. Nine patients died of surgery-related causes (sepsis in five, graft failure in one, multiple organ failure in one, abdominal bleeding in one, and fungal infection in one). The other 14 patients died of medical reasons (HCC recurrence in 4, chronic rejection in 3, HCV recurrence in 2, PSC recurrence in 1, hemangioendothelioma recurrence in 1, fungal infection in 1, adult T-cell leukemia in 1, and subcapsular hemorrhage of the hepatic graft secondary to liver biopsy in 1).

Figure 1 shows the overall patient and graft survival rates among the four groups. The 1-, 3-, 5-, and 10-year patient survival rates in the MM group were 87.0%, 77.7%, 76.2%, and 70.2%, respectively. Those in the female donor to female recipient (FF) group were 94.0%, 84.6%, 81.6%, and 81.6%, respectively. Those in the MF group were 83.3%, 83.3%, 81.7%, and 75.4%, respectively. Those in the FM group were 80.6%, 66.8%, 61.8%, and 47.7%, respectively. The FM group had significantly worse patient survival rates compared with the FF ($P<0.01$), MF ($P<0.01$), and MM ($P<0.05$) groups.

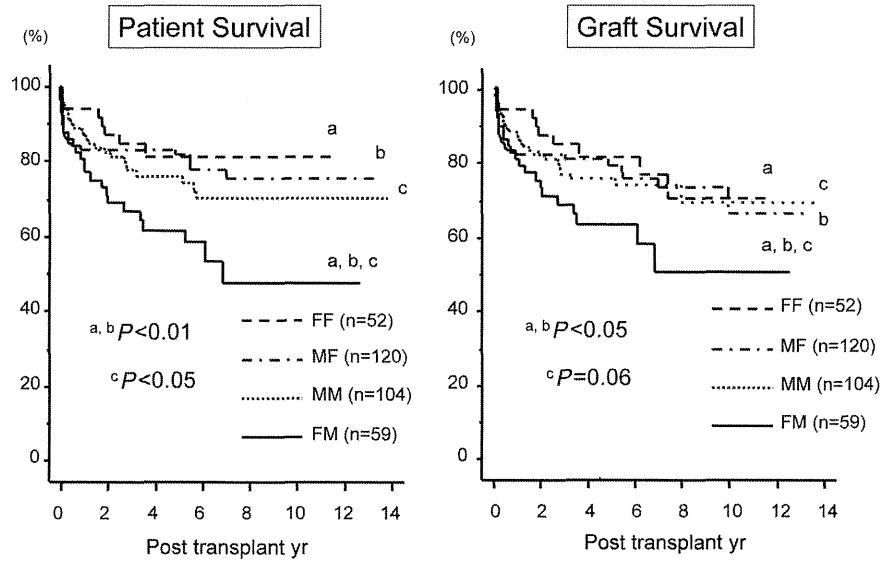

FIGURE 1. Patient and graft survival after LDLT among the four groups defined according to gender combination. The FM group had significantly worse patient survival rates compared with the FF (a, $P<0.01$), MF (b, $P<0.01$), and MM (c, $P<0.05$) groups. The FM group had significantly worse graft survival rates compared with the FF (a, $P<0.05$) and MF (b, $P<0.05$) groups. FM, female donor to male recipient; FF, female donor to female recipient; MF, male donor to female recipient; MM, male donor to male recipient; LDLT, living donor liver transplantation.

The 1-, 3-, 5-, and 10-year graft survival rates in the MM group were 86.2%, 76.9%, 75.5%, and 65.4%, respectively. Those in the FF group were 94.1%, 84.7%, 81.6%, and 70.4%, respectively. Those in the MF group were 81.2%, 81.2%, 78.4%, and 65.8%, respectively. Those in the FM group were 80.9%, 67.0%, 62.0%, and 47.1%, respectively. The FM group had significantly worse graft survival rates compared with the FF ($P<0.02$) and MF ($P<0.05$) groups.

Univariable analysis revealed the following risk factors for patient mortality after LDLT: MELD score more than or equal to 20; the presence of pretransplant diabetes mellitus (DM); absence of consanguinity between the donor and recipient; and inclusion in the FM group (Table 2). The following variables had P values of less than 0.10: donor age more than 60 years and liver failure without HCC. A multivariable analysis including these variables revealed that the FM group ($P=0.006$), the presence of DM ($P=0.001$), and a MELD score more than or equal to 20 ($P=0.004$) were independent risk factors for patient mortality after LDLT (Table 3). Figure 2 shows the overall patient survival rate according to each risk factor.

DISCUSSION

This is the first report on the impact of gender and LDLT outcomes. That a multivariable analysis identified organ donation from a female to a male recipient as an independent risk factor for patient mortality after LDLT is of interest. Because the male–female difference in body size is believed to be a factor in lower survival rates, we performed a subgroup analysis in the FM group according to the GW-SLW ratio. Patients were classified into two subgroups: those with a GW-SLW ratio of less than 40% (n=26) and those with a GW-SLW ratio of more than or equal to 40% (n=33). Findings showed no significant differences between the subgroups (data not shown). Recipient age and primary diagnosis, such as HCV or fulminant hepatic failure, also had no effect on outcomes (data not shown). Biliary issues (type of reconstruction and stricture), which are likely important and related to infection complications, were assessed in the univariable analysis. They did not affect outcomes either (Table 2).

When using a right lobe graft, complicated reconstruction of the middle hepatic vein (MHV) is necessary (*12*). Therefore, the FM group had the highest operation time, cold ischemic time, and recipient blood loss (Table 1). The prevalence of a complicated operation for right lobe graft might have affected outcomes in this study. The patency rate of these reconstructed veins confirmed by Doppler echo 7 days after LDLT was 80%. The frequency of SFSG syndrome in each group was assessed. A total of 30 of 120 cases using a right hepatic graft developed SFSG syndrome. Among them, 10 were in the MF group (47.6%), 12 were in the FM group (27.9%), 5 were in the FF group (20.8%), and 3 were in the MM group (9.7%). The difference was significant ($P=0.02$); however, the frequency rate of SFSG syndrome was more in the MF group than in the other three groups.

In this study, the prevalence of DM, which is a well-accepted risk factor for mortality in cadaveric LT (*14*), differed between males and females (Table 1). Furthermore, the presence of DM was an independent risk factor for mortality.

In the FM group, 44% of recipients received grafts from nonconsanguineous donors (spouses). Univariable analysis revealed that a lack of consanguinity between donor and recipient was a risk factor for mortality, although the frequency of acute cellular rejection did not differ among the four groups. Therefore, it is not clear how consanguinity affected outcomes in this study.

Although Marino et al. (*15*) reported that livers from female donors yielded poorer results even in female recipients, perhaps because of a gender-related immunologic factor or sex hormones, this study confirmed that the FF group had the best patient survival (Fig. 1). It also showed that having a female donor was not a risk factor for survival.

Outcomes from this study are somewhat consistent with those of prior reports on cadaveric LT (*6, 8*). The increased risk of graft failure in male recipients of female livers may be related to the lack of estrogen and/or progesterone in male recipients (*16*). Furthermore, the human liver has gender-related differences, such as increased hepatic content of microsomal oxidative enzymes in males and different

TABLE 2. Risk factors for patient survival after LDLT: univariable analysis

Variables	Patient survival 1 yr	3 yr	5 yr	P
Recipient variables				
Age (%)				
≥60 yr (n=84)	85.4	75.4	73.4	NS
<60 yr (n=251)	85.7	79.5	77.0	
Etiology (%)				
HCV (n=147)	87.7	77.5	75.3	NS
Others (n=188)	84.0	79.4	76.9	
HCC (%)				
No (n=180)	80.5	74.7	73.0	0.077
Yes (n=155)	91.7	83.2	80.0	
MELD score (%)				
≥20 (n=72)	72.2	65.7	63.3	0.003
<20 (n=258)	89.1	82.4	80.0	
Diabetes mellitus (%)				
Yes (n=51)	70.9	63.9	61.0	0.005
No (n=284)	88.3	81.3	79.0	
Bile duct reconstruction (%)				
Roux-en-Y (n=81)	82.3	76.6	74.9	NS
Duct to duct (n=251)	87.0	79.6	76.9	
Bile duct stenosis (%)				
Yes (n=71)	94.3	83.6	80.3	NS
No (n=262)	83.8	78.0	75.9	
Donor/graft variables				
Age (%)				
≥60 yr (n=6)	66.7	66.7	66.7	0.089
<60 yr (n=329)	86.0	78.9	76.5	
Graft (%)				
Left lobe (n=210)	84.2	80.0	77.9	NS
Others (n=125)	88.2	76.4	73.4	
GW-SLW ratio (%)				
≤35 (n=68)	83.4	77.7	75.4	NS
>35 (n=264)	86.1	78.8	76.3	
Donor-recipient matching				
ABO incompatible (%)				
Yes (n=17)	86.2	86.2	86.2	NS
No (n=318)	85.5	78.3	75.9	
Consanguinity (%)				
No (n=55)	80.9	68.6	68.6	0.030
Yes (n=280)	86.6	80.6	77.8	
Donor-recipient gender (%)				
Mismatch (n=179)	82.4	77.6	74.9	NS
Match (n=156)	89.3	79.9	78.0	
FM group (%)				
Yes (n=59)	80.6	66.8	61.8	0.002
No (n=276)	86.7	81.3	79.5	

LDLT, living donor liver transplantation; GW, graft weight; SLW, standard liver weight calculated by 706.2×body surface area+2.4; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; NS, not significant; MELD, model for end-stage liver disease; FM, female donor to male recipient.

TABLE 3. Risk factors for patient survival after LDLT: multivariable analysis

Variables	Odds ratio	95% CI	P
FM group			
Yes vs. No	2.10	1.24–3.57	0.006
Diabetes mellitus			
Yes vs. No	2.76	1.56–4.88	0.001
MELD score			
≥20 vs. <20	2.12	1.27–3.53	0.004
Donor age (yr)			
≥60 vs. <60	2.79	0.85–9.17	0.09
HCC			
No vs. yes	1.54	0.90–2.64	0.11
Consanguinity			
No vs. yes	1.37	0.77–2.43	0.28

LDLT, living donor liver transplantation; CI, confidence interval; FM, female donor to male recipient; MELD, model for end-stage liver disease; HCC, hepatocellular carcinoma.

numbers of estrogen and androgen receptors on hepatocytes between males and females (*7*).

In a rodent hepatectomy model, serum estrogen levels and the number of estrogen hepatic receptors increased concomitantly with liver regeneration (*17*). Kahn et al. (*18*) also demonstrated a reduction in the number of estrogen receptors in the livers of gender-mismatched recipients 10 days after transplantation. Thus, it is possible that the poor outcome in the FM group was caused by reduced serum estrogen levels in the male recipients and a lower number of estrogen receptors in the female organ. Further long-term study is warranted to clarify how hormonal factors affect the outcome of LT.

Because of the shortage of donor organs, the gender of donors is not routinely used as a selection criterion for LDLT (*19*). Although LDLT allows for elective planning of the procedure, which may enable selection of the most suitable donor from among the candidates (*19*), it is important to be mindful of hormonal and/or immunological differences between the genders to improve LDLT outcomes. At the same time, we need to remember that a multiplicity of donor and recipient factors influence posttransplant outcomes (*20*). For these reasons, further study in this area is called for before any changes in clinical decision-making based on findings in this report.

In conclusion, male recipients who received transplants from female donors had the worst survival among the four donor-recipient groups. Being a male recipient receiving a transplant from a female donor was an independent risk factor for patient mortality after LDLT. Further study is warranted to clarify the mechanism of this outcome.

MATERIALS AND METHODS

Patients

A total of 335 adult patients (172 women and 163 men) who had undergone LDLT because of end-stage liver disease at Kyushu University Hospital between May 1997 and March 2011 were enrolled in the trial; seven retransplanted cases were included. The cause of liver disease (women/men) was hepatitis C (59/88), fulminant hepatic failure (32/18), primary biliary cirrho-

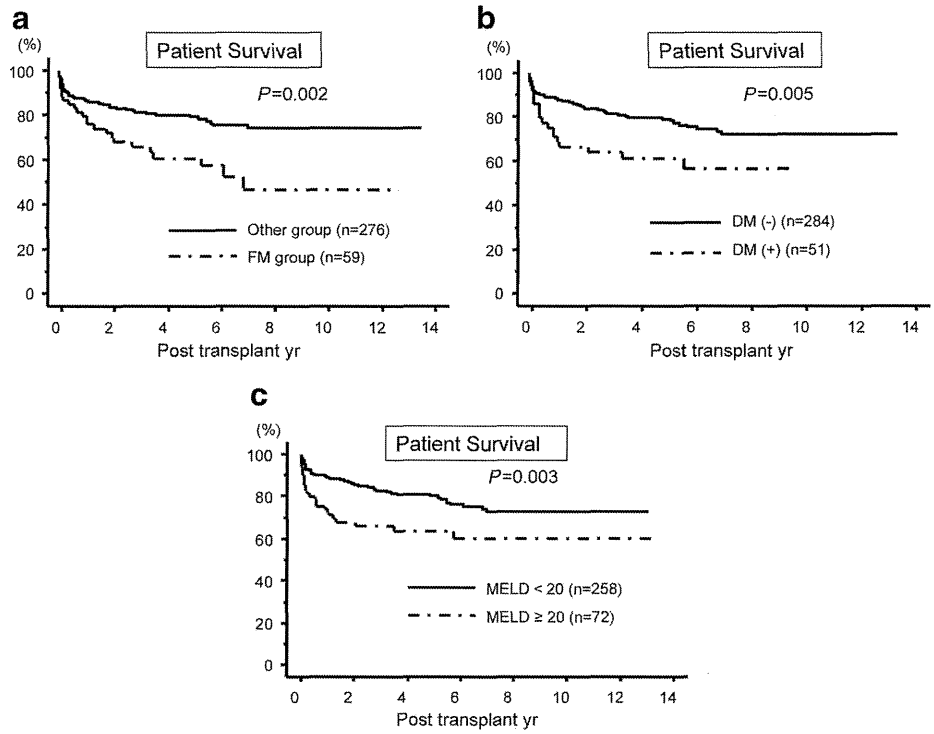

FIGURE 2. (a) Patient survival after LDLT between the two groups according to gender combination. The 1-, 3-, 5-, and 10-year patient survival rates in the FM group were 80.6%, 66.8%, 61.8%, and 47.7%, respectively, and those in the other combination groups were 86.7%, 81.3%, 79.5%, and 74.5%, respectively. 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

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

Yusuke Arakawa · Mitsuo Shimada · Tohru Utsunomiya · Satoru Imura · Yuji Morine · Tetsuya Ikemoto · Jun Hanaoka

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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.

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

Y. Arakawa (✉) · M. Shimada · T. Utsunomiya · S. Imura · Y. Morine · T. Ikemoto · J. Hanaoka
Department of Surgery, Institute of Health Bioscience, The University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan
e-mail: yarakawa@clin.med.tokushima-u.ac.jp

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(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).

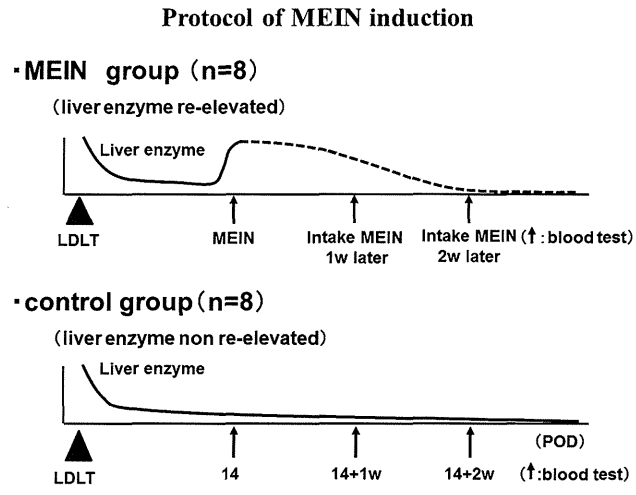

Fig. 1 The timing of the re-elevation of liver enzyme levels and the administration of MEIN

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.

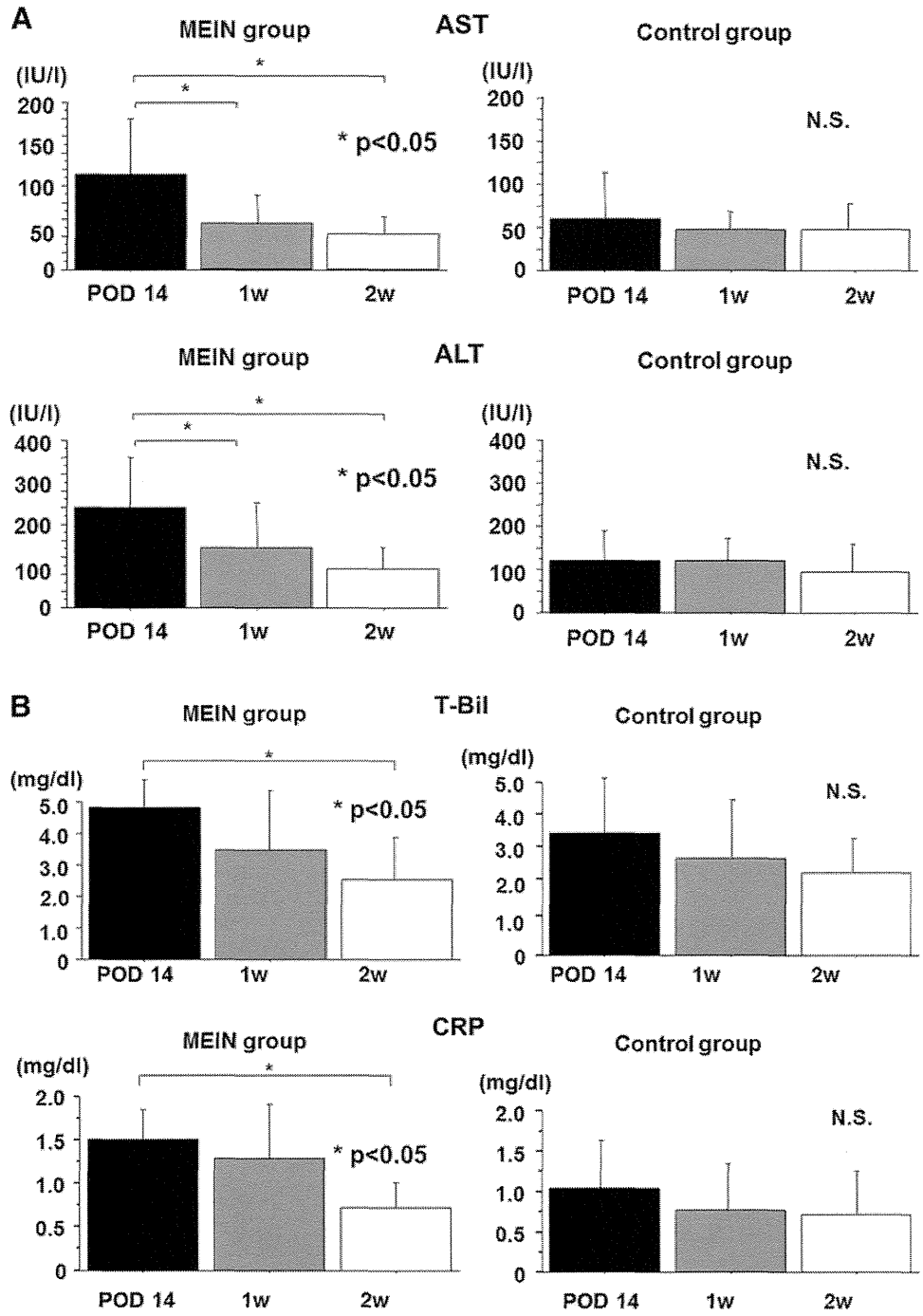

Fig. 2 The results of the biochemical analyses of the patients in the MEIN and control groups. **a** Aspartate aminotransferase (AST) and alanine aminotransferase (ALT), **b** total bilirubin (T-Bil) and C-reactive protein (CRP) levels

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.

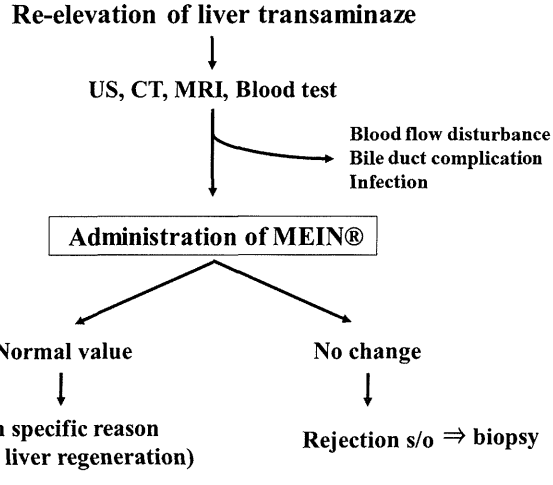

Fig. 3 A proposed flow chart of the postoperative management of patients who show a re-elevation of AST and ALT after LDLT

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

Yusuke Arakawa, M.D., Mitsuo Shimada, M.D., F.A.C.S.,[2] Tohru Utsunomiya, M.D., Satoru Imura, M.D., Yuji Morine, M.D., and Tetsuya Ikemoto, M.D.

Department of Surgery, Institute of Health Biosciences, The University of Tokushima, Tokushima, Japan

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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.**

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

[1] A part of this study was presented in ASCO-GI, San Francisco, January 16–18, 2009.

[2] To whom correspondence and reprint requests should be addressed at Institute of Health Biosciences, Department of Surgery, The University of Tokushima Graduate School, 3-18-15 Kuramoto, Tokushima, 770-8503 Japan. E-mail: mshimada@clin.med.tokushima-u.ac.jp.

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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 $\times$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 $\pm$ 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