polymerase chain reaction (PCR). The genomic DNA isolated from the homogenate of a grafted liver biopsy specimen and intestinal mucosa or peripheral blood from recipients was used for genotyping by the PCR restriction enzyme length polymorphism (RFLP) method.[2] This study was conducted in accordance with the Declaration of Helsinki and its amendments, and was approved by the ethics committee of Kyoto University.

Normally distributed values were presented as the mean SE. Logarithmic transformation of the mRNA expression levels of MDR1 and CYP3A4 was performed to improve normality before performing statistical analyses. The unpaired Student's *t* test was used to compare groups with respect to normally distributed variables.

RESULTS AND DISCUSSION

Although the tacrolimus dosage regimen for each recipient was defined as the daily trough level of tacrolimus from day 2 in each recipient, half of the tacrolimus trough levels were below the lower limit of the therapeutic range, which was set at 10 to 20 ng/mL, during 7 postoperative days (90 of 201 measurements, n 46).

The quantified average expression levels of MDR1 mRNA and CYP3A4 mRNA in all 48 intestinal mucosa obtained was 0.41 1.19 and 1.58 1.25 (micromoles per gram total RNA; mean SE), respectively.[3] To clarify whether MDR1 and CYP3A4 play roles as pharmacokinetic factors for tacrolimus therapy after LDLT, we examined the pharmacokinetic significance of these absorptive barriers with the tacrolimus C/D ratio in LDLT recipients. The logarithmically transformed mRNA expression level of MDR1 (r 0.776), but not CYP3A4 (r 0.096), was inversely correlated with the C/D ratio of tacrolimus administered orally. Moreover, we categorized recipients by mRNA expression levels of MDR1 and CYP3A4 on the basis of each mean value. The differences in dose, the trough level, and the C/D ratio were examined between the high and low groups for both MDR1 and CYP3A4. The tacrolimus trough level did not differ significantly between the two MDR1 groups (11.5 0.58 in the high-MDR1 group vs 11.0 0.52 ng/mL in the low-MDR1 group, P .476). However, the oral dose and the C/D ratio of tacrolimus in the high-MDR1 group were approximately twofold higher (0.13 0.01 vs 0.07 0.01 mg/kg per day, P .001) and lower (143.8 18.2 vs 230.1 20.9 ng/mL · mg/kg per day P .002) than in the low-MDR1 group, respectively. There was no significant difference between the high- and low-CYP3A4 groups in tacrolimus dose or C/D ratio.

Next, we preliminarily predicted the initial oral dosage of tacrolimus based on the mRNA expression level of intestinal MDR1 mRNA at LDLT. Despite the small number of subjects, the period required for the blood level of tacrolimus to reach therapeutic range (10 ng/mL) was faster in the predicted group (n 27) than in the nonpredicted group (n 35). In addition, the correlation between the intestinal mRNA level of MDR1, and the tacrolimus C/D ratio during 7 postoperative days was confirmed in the additional cases (n 104, r 0.645). These results suggest that the intestinal expression level of MDR1 was a good molecular marker to define the oral dosage regimen of tacrolimus, at least immediately after LDLT.

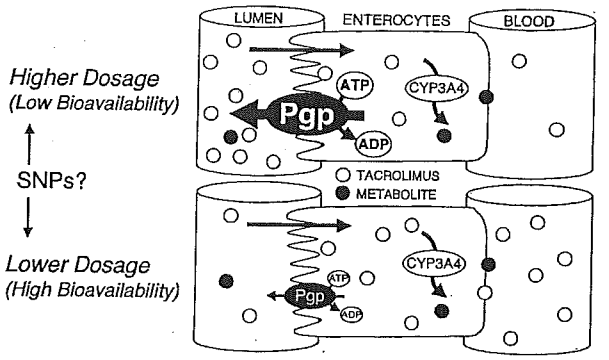

Fig 1. Interindividual variation of tacrolimus absorption. Recipients with a high level of intestinal MDR1 require a higher initial dose of tacrolimus.

Recently, the MDR1 cDNA 3435C/T and 2677G/AT single-nucleotide polymorphisms (SNPs) were reported as factors affecting the intestinal expression and/or functional property of MDR1.[4] However, the frequencies of MDR1 3435C/T and 2677G/AT genotypes were similar to previous reports, but both the tacrolimus C/D ratio during 7 postoperative days and the intestinal mRNA level of MDR1 were not affected by these SNPs.[4,5] Therefore, the intestinal mRNA expression level of MDR1 at LDLT was a potent pharmacokinetic factor without the influence of these two SNPs.

In conclusion, intestinal MDR1 functions as an absorptive barrier for orally administered tacrolimus, and its mRNA level at LDLT could contribute to establishment of an individualized oral dosage regimen of tacrolimus immediately after surgery (Fig 1).

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IMAGES OF INTEREST

Hepatobiliary and pancreatic: The color bar sign after living donor liver transplantation

In living donor liver transplantation, the first reported procedures were carried out with a left liver graft. Subsequently, better results were achieved with a right liver graft, particularly when the recipient was an adult. One technical issue involves the venous drainage of the right graft that normally includes the right hepatic vein and tributaries of the middle hepatic vein. In order to avoid venous congestion of the right anterior section of the graft, remnant tributaries of the middle hepatic vein are often anastomosed to the right hepatic vein. The challenge is to document these anastomoses in the postoperative setting, when it might be difficult to distinguish branches of the portal vein from branches of the middle hepatic vein.

We have used color Doppler ultrasonography to assess different features of the vasculature after living donor liver transplantation. The portal vein accompanies the hepatic artery, although it is often difficult to detect the hepatic artery on the periphery of the liver. Furthermore, blood flow velocity in the portal vein decreases towards the liver periphery, whereas blood flow velocity in the drainage veins (right hepatic vein and tributaries of the middle hepatic vein) increases towards the liver periphery. These features are shown in Fig. 1. The remnant tributary of the middle hepatic vein is shown with solid arrows, whereas open arrows are used for the right hepatic vein. The curved arrow indicates the direction of blood flow. The increasing blood velocity in the remnant tributary of the middle hepatic vein mimics the upper side of a color bar; and hence it is called the color bar sign. Pulsed Doppler ultrasonography can also be used to confirm the drainage vein by analysis of the waveform. The phasic waveform associated with the tributary of the middle hepatic vein is shown in Fig. 2. This new sign might be useful for the diagnosis of functional anastomoses in recipients of a right liver graft.

Contributed by
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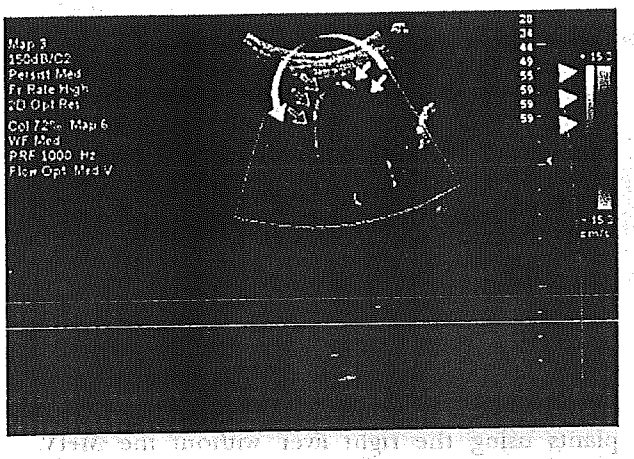

Figure 1

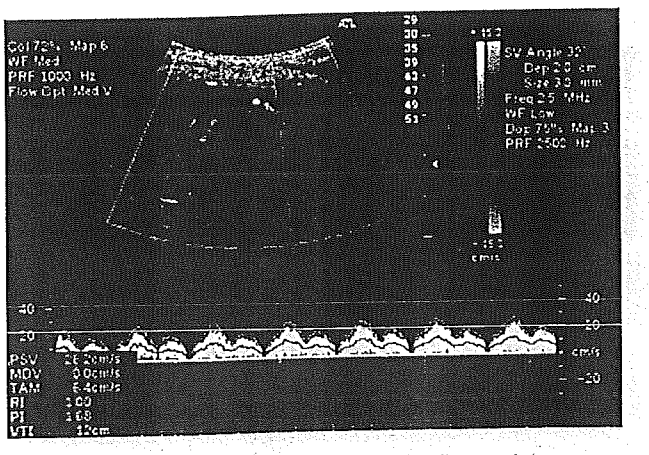

Figure 2

Contributions to the Images of Interest Section are welcomed and should be submitted to Professor IC Roberts-Thomson, Department of Gastroenterology, The Queen Elizabeth Hospital, Woodville South, South Australia 5011, Australia.

Intrahepatic venous anastomosis formation of the right liver in living donor liver transplantation: Evaluations by Doppler ultrasonography and pulse-inversion ultrasonography with Levovist

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Background. *Our aim was to investigate the development of intrahepatic venous anastomoses between the middle hepatic vein (MHV) and the right hepatic vein (RHV) in adult-to-adult, living donor, liver transplantation.*

Methods. *Using Doppler ultrasonography, we studied the formation of venous anastomoses between the MHV tributaries for segments 5 and 8 (V5, V8) and the RHV in the liver remnants of 7 donors of a left liver, including the MHV, and in the liver grafts of 8 recipients of a right liver, without including the MHV. In 1 donor and 5 recipients, we performed pulse-inversion ultrasonography with a microbubble contrast agent to evaluate hepatic parenchymal perfusion in the drainage region of the MHV.*

Results. *We observed 15 MHV tributaries of V5 and 13 of V8 among the 15 adult transplant patients. During the first postoperative week, we detected venous anastomosis between V5 and the RHV in 4 patients and in 10 patients between V8 and the RHV. After the 1st week, we observed the formation of anastomosis between V5 and the RHV in 10 patients, and between V8 and the RHV in 3. In both MHV tributaries, the mean flow velocities increased* ($P < .01$). *By the end of the 1st week, the formation rate in V8 was higher than in V5 (77% vs 27%,* $P < .03$). *In the parenchymal phase of the pulse-inversion ultrasonography with the microbubble contrast agent, the V5 drainage region had low intensities, while the V8 drainage territory revealed high intensities in 4 of 6 patients (66.7%).*

Conclusions. *Functional venous anastomoses between either V5 or V8 and the RHV developed in most of the donors of left hepatic lobes and in recipients of right hepatic lobes; however, anastomoses developed earlier in V8 than in V5. Furthermore, perfusion was decreased in the drainage area of V5, compared with V8. (Surgery 2005;138:21-7.)*

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LIVING DONOR liver transplantation has become used widely in the adult patient population.[1,2] The use of a right liver graft without including the middle hepatic vein (MHV) has become a standard option to ensure adequate hepatic graft volumes capable of fulfilling the metabolic demands of recipients.[3-5] The right anterior (segments 5 and 8) of the liver is drained primarily by the MHV.[6] As a result, drainage of the right anterior sector in right liver grafts without the MHV drainage is critically dependent on the integrity of intrahepatic venous anastomoses between the MHV tributaries and the right hepatic vein (RHV).[7]

According to the Kyoto group, graft loss consequent to graft congestion did not occur among their series of more than 200 living donor transplants using the right liver without the MHV.[8] Conversely, Lee et al[9] reported severe congestion of the anterior sector of right liver grafts without the MHV after reperfusion, which resulted in severe graft dysfunction and septic complications. They now reconstruct all the MHV tributaries for the right anterior sector of right liver grafts when the MHV is not included in the graft. It remains

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Table I. Demographic data

Patient total	*n = 15*
Recipient age (y)	43 ± 16 (17-60)
Recipient gender (M/F)	8/7
Donor age (y)	38 ± 12 (21-56)
Donor gender (M/F)	10/5
Diseases	
Hepatitis-induced liver cirrhosis	5
Primary biliary cirrhosis	4
Biliary atresia	2
Familial amyloid polyneuropathy	2
Primary sclerosing cholangitis	1
Fulminant hepatic failure	1
Operative procedures of donor	
Left hepatectomy (segments 2, 3, and 4 with MHV)	7
Right hepatectomy (segments 5, 6, 7, and 8 without MHV)	8

MHV, Middle hepatic vein.

controversial as to whether the MHV tributaries should be reconstructed and whether a single hepatic vein can provide drainage for the entire right liver graft, presumably through the formation of intrahepatic venous anatomoses.[10]

In patients in whom the MHV tributaries are not reconstructed in a right liver graft, the integrity of the intrahepatic venous anastomoses between the MHV and RHV is of considerable importance because there is no other collateral route for blood to exit the liver. Compromise of these anastomoses could lead to venous outflow obstruction, causing graft congestion and potential graft failure. Our aim was to assess the intrahepatic formation of venous anastomoses between the MHV and RHV, utilizing color Doppler ultrasonography (US). Doppler US has been used extensively in the evaluation of liver grafts and hepatic vasculature. The intrahepatic hemodynamics is similar in recipients of a right liver graft without the MHV and in the right liver remnant in donors of a left liver graft with the MHV. As a result, we evaluated formation of venous anastomoses between the MHV tributaries and the RHV in recipients of right liver grafts without the MHV and in donors of left liver grafts with the MHV. We also utilized Pulse-inversion harmonic US with a microbubble contrast agent to assess hepatic parenchymal perfusion as another measure of the development of intrahepatic venous collaterals.[11,12] This technology results in improved spatial and temporal resolution in comparison with conventional Doppler US, as well as in heightened contrast compared with harmonic US.[13]

In this paper, we use the nomenclature specified by the Hepato-Pancreato-Biliary Association regarding the anatomic terminology of the liver.[14]

PATIENTS AND METHODS

Patients. We studied 15, adult-to-adult, living donor liver transplantation procedures. The demographic data of the donors and recipients are listed in Table I. The operative procedures consisted of a left hepatectomy (segments 2, 3, and 4 with the MHV) in 7 patients and a right hepatectomy (segments 5, 6, 7, and 8 without the MHV) in 8 patients. Detailed descriptions of right liver graft recipients, including liver diagnoses and relative sizes of the liver grafts, are shown in Table II. In the right liver grafts, we did not reconstruct the MHV tributaries from the right anterior sector (V5, V8). In the left liver donors, we kept the right livers without the MHV and ligated the MHV tributaries from the right anterior sector. In both groups, the MHV outflow pathway for the right anterior sector was interrupted.

Written informed consent was obtained from all patients in this study.

Intra- and postoperative Doppler US examination of hepatic venous flow. We performed pulse and color Doppler US to measure flow of the MHV tributary from segment 5 (V5) and segment 8 (V8), as well as flow of the portal vein branch to segment 5 (P5) and segment 8 (P8). We performed intraoperative Doppler US examinations and investigated the presence of intrahepatic venous anastomoses between either V5 or V8 and the RHV. All Doppler US exams were performed by transplant surgeons qualified in sonography (T.K., H.S.). Postoperative US examinations were performed by right intercostal scanning during expiration with the use of a commercially available US machine (HDI 5000; Philips, Andover, Mass). The highest-frequency, curved array US transducer that could resolve the Doppler signals was used, namely the C5-2. The sample volume was set at 2.5 mm. So far as was tolerated by the patient, the portal vein flow was investigated fully up to the 3rd-ordered branch. Flow of the ventral branch of P8 was measured. During all intraoperative US exams, a 5 to 8 MHz transducer with a microconvex probe was used; postoperatively, a 2 to 5 MHz transducer with a convex probe was utilized. Among the recipients, we performed Doppler US twice a day postoperatively until postoperative day (POD) 14. Subsequently, Doppler US was performed once a day until POD 28 and then weekly thereafter. Among the donors, we performed Doppler US

Table II. Recipients of right liver grafts

	Liver disease	*Age (y)*	*Gender*	*GV/SLV × 100 (%)*
1	Biliary atresia	16	M	56
2	Primary biliary cirrhosis	59	F	81
3	Familial amyloid polyneuropathy	30	M	45
4	Hepatitis-induced liver cirrhosis	59	M	79
5	Hepatitis-induced liver cirrhosis	58	M	61
6	Hepatitis-induced liver cirrhosis	52	M	52
7	Primary biliary cirrhosis	56	F	56
8	Hepatitis-induced liver cirrhosis	60	M	49
				60 ± 13

GV, Graft volume; *SLV,* standard liver volume.

once a day until POD 14 and then twice a week until POD 28.

The criteria for the presence of a collateral on Doppler US was reversed flow in the ligated MHV tributary toward the periphery that then communicated with the RHV. Reversed flow of the ligated MHV tributary increased toward the intrahepatic anastomoses in a pattern of flow different from the portal vein. This phenomenon has been reported as a "color bar sign," which mimics the color bar at the right side of the color Doppler image.[15]

Pulse-inversion harmonic US with Levovist. We performed a Pulse-inversion harmonic US with Levovist (Schering, Berlin, Germany), a microbubble contrast agent for US between POD 21 and 54 (average, 25 POD) to evaluate hepatic parenchymal perfusion. The Pulse-inversion mode US with Levovist was also performed by transplant surgeons qualified in sonography (T.K., H.S.). We used a commercially available US machine (HDI 5000; Philips) with a Pulse-inversion mode installed on a 2 to 5 MHz transducer with a convex probe. We preset the acoustic power at a mechanical index (MI) of 1.5. We then administered 2.5 g of Levovist as an intravenous bolus (concentration, 300 mg/mL), followed by 10 mL of 154 mmol/L NaCl through an 18-gauge peripheral venous cannula. After 5 minutes, we scanned the entire liver in the Pulse-inversion mode during a breath-hold. The focal zone was set at the shallow one third of the image to evaluate hepatic parenchymal perfusion, particularly in segment 5.

Pulse-inversion harmonic US is a technique capable of detecting nonlinear echoes. Alternative pulses, 180 out of phase, are transmitted along a given ultrasound line. The signals from the pair are summed and used to form 1 image line. Signals from linear and nonlinear sources can be differentiated in the receiving spectrum because nonlinear signals summate and linear signals cancel.

Statistical analysis. All results were expressed as the mean + SD. Continuous variables were evaluated with repeated measures of analysis of variance (ANOVA), followed by a Bonferroni correction. We used Fisher exact test to compare discrete variables and obtained the correlation coefficient using Spearman rank correlation analysis. Differences with a P value less than .05 were considered statistically significant.

RESULTS

Doppler US examination. We detected formation of intrahepatic venous anastomoses between V5 or V8 and the RHV by color and pulse Doppler US. Color Doppler US demonstrated reversal of venous flow in V5 and V8 into the RHV by development of intrahepatic venous anastomoses. Pulse Doppler US showed mirror image, waveform patterns in V5 and the RHV at the site of the anastomosis (Fig 1).

MHV tributary from segment 5. We evaluated 15 tributaries of V5, identifying 1 tributary in each patient. We found intrahepatic venous anastomoses between V5 and the RHV in 1 patient on POD 1, collateral formation in 3 patients between PODs 2 and 7, in 5 patients between PODs 8 and 14, and in another 5 patients after POD 15. We did not detect any anastomoses in 1 patient. During the first postoperative week, 4 of the 15 patients developed venous anastomoses between V5 and the RHV. After the 1st week, 10 of the 15 patients developed collaterals.

The mean flow velocities of V5 were 1.2 + 4.6 cm/s on POD 1, 4.2 + 7.6 cm/s between PODs 2 and 7, 5.5 + 6.6 cm/s between PODs 8 and 14, and 9.7 + 5.8 cm/s after POD 15. These velocities gradually increased over time ($P < .01$). The mean velocities of PODs 8 through 14 and after POD 15 were greater than those of POD 1 ($P < .05$ and $P < .01$, respectively).

MHV tributary from segment 8. Thirteen tributaries for segment 8 were evaluated. In 2 patients, V8 could not be detected by postoperative Doppler US examination. We were able to detect intrahepatic venous anastomoses between V8 and the RHV in 4 patients on POD 1, in 6 patients between PODs 2 and 7, in 1 patient between PODs 8 and 14, and in 2 patients after POD 15. During the 1st week, 10 of the 13 patients developed

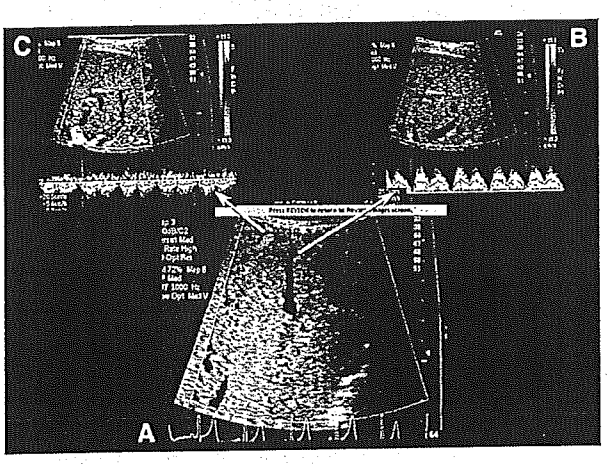

Fig 1. A, Color Doppler US demonstrating intrahepatic venous anastomosis between the MHV tributary (V5) and the RHV. **B,** Pulsed Doppler US at V5 showing reversed flow toward the anastomosis. **C,** Pulsed Doppler US at the RHV showing flow away from the anastomosis.

collaterals, whereas only 23% (3/13) of the patients formed collaterals after the 1st week.

The mean flow velocity of V8 on POD 1 was 3.5 + 5.6 cm/s, 8.4 + 6.2 cm/s between PODs 2 and 7, 10.5 + 6.8 cm/s between PODs 8 and 14, and 14 + 6.0 cm/s after POD 15. These velocities also increased gradually ($P < .01$). The mean velocities of PODs 2 through 7, PODs 8 through 14, and after POD 15 were greater than those of POD 1 ($P < .01$, respectively).

Portal vein branch to segment 5. We examined 15 portal vein branches to segment 5, investigating 1 branch in each patient. Blood flow of the ventral branch of P5 was measured. Hepatofugal flow was observed in 5 patients on POD 1, in 6 patients between PODs 2 and 7, and in 3 patients between POD 8 and 14. We did not observe hepatofugal venous flow in the other patients after POD 15.

The mean flow velocities of P5 on POD 1, POD 2 through 7, POD 8 through 14, and after POD 15 were 0.2 + 7.9 cm/s, 4.3 + 11.1 cm/s, 6.6 + 9.8 cm/s, and 12.1 + 8.1 cm/s, respectively. The mean velocities increased gradually ($P < .05$). The mean velocities after POD 15 were greater than those of POD 1 ($P < .01$).

Portal vein branch to segment 8. We examined 15 portal vein branches to segment 8, evaluating 1 branch per patient. Blood flow of the ventral branch of P8 was measured. Hepatofugal flow was observed in 1 patient between PODs 2 and 7. The mean velocities of P8 on POD 1, PODs 2 through 7, PODs 8 through 14, and after POD 15 were 6.3 + 7.4 cm/s, 7.3 + 7.8 cm/s, 9.4 + 7.6 cm/s, and 12.9 + 6.3 cm/s, respectively. There was no statistical difference in velocity over time.

Comparison of intrahepatic anastomosis formation between V5 and V8. The rate of formation of intrahepatic venous anastomoses during the 1st postoperative week was greater in V8 than in V5 (77% vs 27%, $P < .03$). Subsequently, the rate of formation of anastomoses after the 1st week was greater in V5 than in V8 (68% vs 23%, $P < .03$). These findings indicate that venous collaterals formed earlier in V8 than in V5.

Comparison of mean velocity between V5 and V8 in each POD group. In every postoperative period, the mean velocity of V8 was not statistically different from that in V5.

Correlation between V5 and P5 and between V8 and P8. The gradual increase in mean velocities over time for V5 and P5—and for V8 and P8—was correlated closely ($r = 0.935$, $P < .01$, and $r = 0.955$, $P < .01$, respectively).

Pulse-inversion harmonic US with Levovist. We performed Pulse-inversion harmonic US in 6 patients (1 donor and 5 recipients) at varying postoperative dates. In 4 patients, the drainage region of V5 was visualized at low signal intensity during the hepatic parenchymal phase, whereas the region of V8 showed high signal intensity (Fig 2). These findings suggest that perfusion of hepatic parenchymal was occurring in segment 5 in comparison to segment 8 in these patients. In the remaining 2 patients, the drainage region of V5 was visualized at high signal intensity, concomitant with the high intensity of segment 8.

Clinical outcome. The postoperative courses of the 7 donors of a left liver graft were uneventful; all patients were discharged within 2 weeks. The postoperative courses of 5 of the 8 right liver graft recipients were also uneventful. Three recipients died after transplantation between PODs 12 and 153, secondary to acute rejection, graft-versus-host disease, and exacerbation of hepatitis C virus infection. No sign of acute congestion or acute hepatic dysfunction was present in this study.

DISCUSSION

In hepatic surgery, ligation of the hepatic venous tributaries is not thought to compromise liver function because of the presence of multiple intrahepatic venous communications between adjacent hepatic veins.[16] After ligation of hepatic veins, areas of potential venocongestion can contribute to the dysfunctional volume of a remnant liver. In a right liver graft without the MHV, it remains controversial as to whether adequate venous drainage of the right anterior sector can be provided by a single hepatic vein in concert with the development of

intrahepatic venous anastomoses between the MHV and RHV.[17,18] A consensus does not yet exist regarding the optimal strategy for venous reconstruction of the MHV tributaries.

Couinaud and Nogueira[19] demonstrated hepatic venous communication in 25 of 30 liver casts by injecting vinyl polychloride into the hepatic veins of autopsied livers. Lasinski and Zientarski[20] found venous anastomosis between the MHV and RHV in half of the examined cases. Nevertheless, these venous anastomoses may not always function immediately after occlusion of the hepatic veins.

In this study, we used color Doppler US to investigate the formation of intrahepatic venous anastomoses between the MHV tributaries and the RHV, as well as the portal venous flow of the right anterior sector. We found that most of the MHV tributaries had developed intrahepatic anastomoses with the RHV after POD 14 during the latter stages of observation (94% in V5 and 100% in V8). Nevertheless, the rate of development of the collaterals differed between the 2 MHV tributaries. During the 1st postoperative week, the intrahepatic venous anastomoses formation rate of V8 was 77% and 27% for V5. In contrast, after the 2nd week, the anastomosis formation rate for V8 was 23%, whereas the rate for V5 was 68%. These results indicate that anastomoses with the RHV develop earlier in V8 than in V5. This finding complements Couinaud's anatomic study of liver casts.[21] He described a prominence of intrahepatic venous anastomoses between the MHV and RHV, especially in segment 8. This finding can be understood from an anatomic standpoint given that the distance between V8 and the RHV in segment 8 is shorter than that between V5 and RHV. The difference in the rate of formation of collaterals between V5 and V8 seems to be therefore a reasonable finding. The mean flow velocity in V8 did not differ from that in V5.

Development of venous anastomoses is influenced invariably by the pattern of venous drainage of the right liver graft. Substantial anatomic variations in the venous drainage of the anterior section have been found. Segment 5 is drained exclusively by the MHV, whereas the dorsal portion of segment 8 is drained reportedly by the RHV in 28% to 35% of patients.[21,22] The grafts in these cases were RHV dominant, and the MHV tributary from the ventral portion of segment 8 was considered to communicate more easily with the RHV.

In this study, hepatofugal venous flow in the portal branch was observed in 6 of 15 livers of P5 during the 1st week after transplantation. In contrast, hepatofugal flow was observed in only 1 of 13 livers of P8 during the 1st postoperative week. The reversal of portal branch venous flow was thought to have impaired the liver regeneration. According to Maema et al,[23] right liver grafts without the MHV had impaired volume regeneration of the right anterior sector when compared with grafts with the MHV preserved. Volume regeneration between segment 5 and segment 8 was not, however, investigated by this group. On the basis of our findings, impaired volume regeneration appears to have been caused primarily by altered venous flow in segment 5. Furthermore, the mean velocity of V5 and V8 correlated closely with the mean velocity of P5 and P8. Consequently, a deficiency of portal venous blood flow in the interrupted venous portion during the postoperative period may be an important factor in compromising the regeneration of the right anterior sector without the MHV.[24] We based this deduction on our long-term postoperative findings.

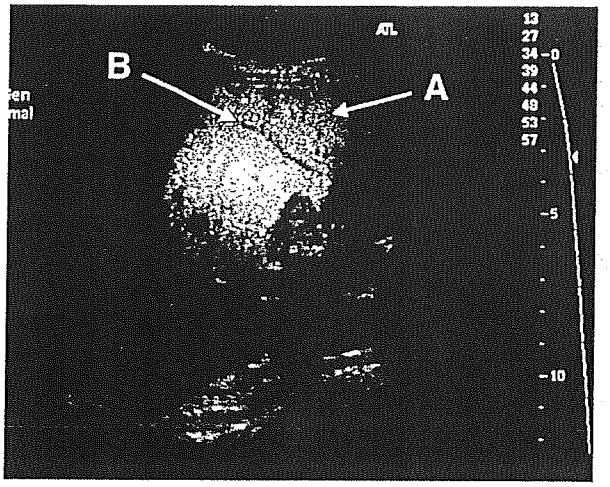

Fig 2. Pulse-inversion harmonic US with Levovist in the liver parenchymal phase of 1 recipient on POD 54. Perfusion in V5 drained area (**A**) was reduced.; V5 (**B**).

In our Doppler US examinations, we investigated the portal venous blood flow up to the 3rd-ordered branch as far as patients could tolerate. In some patients, however, we may have measured proximal portal branch flow that included the well-drained area of segment 8 and the venoocclusive area. Using Doppler US, we found a close correlation between the mean flow velocity of hepatic venous branches and the portal venous branches. Although the points at which these velocities were measured corresponded well between these 2 systems, they were not matched completely with regard to vessel proximity. Nevertheless, the correlation coefficient between mean values was

quite high ($r > 0.9$). This finding warrants further investigation, using a larger number of patients.

The study using pulse-inversion harmonic US revealed decreased hepatic parenchymal perfusion in segment 5 in 4 of 6 of patients, compared with segment 8. This finding suggests that the functional anastomoses between V5 and RHV were less effective than those between V8 and RHV. Even though the number of patients studied was small and the date of the studies varied, we were able to detect a difference in hepatic parenchymal perfusion using a new technique involving Pulse-inversion mode US with Levovist. Furthermore, we consider our findings on hepatic perfusion to be preliminary, given that our initial intention was aimed at compiling data for a large number of patients undergoing pulse-inversion harmonic US studies with Levovist after receiving a living donor liver transplant.

In the acute phase after transplantation, transient congestion of the right anterior sector has been reported.[25] Fortunately, in our small series, we did not notice any patients with severe congestion of the right anterior sector. The degree to which potential congestion of the right anterior sector causes graft dysfunction is difficult to predict. As a result, a large graft size is preferable in patients undergoing living donor liver transplantation for liver cirrhosis. We encountered 2 patients in whom intrahepatic venous anastomoses in both V5 and V8 occurred only after 2 postoperative weeks. These patients carried the diagnosis of primary biliary cirrhosis and liver cirrhosis secondary to hepatitis B virus infection. Neither patient had obvious signs of hepatic failure, presumably because the graft sizes were sufficiently large (average graft volume/standard liver volume × 100 = 66% [52%-81%]). We also studied 2 patients in whom intrahepatic venous anastomoses in V5 formed after only 2 weeks, yet anastomoses formed in V8 during the 1st week. These 2 patients had biliary atresia and liver cirrhosis secondary to hepatitis C virus infection. Similar to the previous example, no obvious hepatic failure was observed. In the patient with biliary cirrhosis, the graft size was rather small (graft volume/standard liver volume × 100 = 45%). In this latter patient, we suspect that the metabolic function of segment 8 may have compensated for a presumed lack of adequate function of segment 5 during the acute phase of transplantation. These findings suggest that the gradual formation of intrahepatic venous anastomoses over time may preclude or relieve venous outflow obstruction and congestion of the right anterior section.[26] When graft size is small, areas of graft congestion may translate into greater adverse effects on liver function and potentially unfavorable clinical consequences. Presently, it is difficult to determine whether intrahepatic collateral circulation will be adequate during acute decompression of the right anterior sector.[21] Therefore, it is preferable to reconstruct V5 or V8 when the graft size is small. On the basis of this study's finding, we would choose to reconstruct V5 rather than V8 because intrahepatic venous anastomoses were found to develop later in V5 than in V8.

This study has several limitations. We assumed that the intrahepatic hemodynamics and anatomy of the right liver were essentially the same between recipients of a right liver graft without the MHV and donors of a left liver graft with the MHV. One caveat of this assumption is that the right liver remnant of a donor has preserved venous drainage through the short hepatic veins from the caudate lobe, whereas the right liver graft does not. Nevertheless, the right anterior sector is drained primarily by branches of the MHV, and the significance of short hepatic venous drainage in this sector, or lack thereof, has yet to be established. Another qualification of this study is that preexisting portal hypertension in the recipients of the right lobe graft in comparison with healthy left liver donors may have affected the rate and significance of collateral development. This possibility warrants further investigation.

We also made attempts to eliminate differences caused by variations in graft size of the right liver, knowing that the size of right liver grafts tend to vary widely and may alter the development of collaterals. In this study, the smallest percentage of graft weight compared with standard liver volume was 45%. Small-for-size cases did not exist.

The formation of intrahepatic venous collaterals was also undoubtedly regulated by anatomic and mechanical factors. The hepatic venous system is a low-pressure system. Alterations in hepatic parenchymal consistency likely influence intrahepatic venous flow. It is reasonable to hypothesize that complications after transplantation, such as infection and rejection, could influence the formation of intrahepatic venous anastomoses by changing the hepatic parenchyma. These issues warrants further investigation in the future.

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LIVER TRANSPLANTATION 12:140–145, 2006

ORIGINAL ARTICLE

Fatal Graft-Versus-Host Disease after Living Donor Liver Transplantation: Differential Impact of Donor-Dominant One-Way HLA Matching

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Graft-versus-host disease (GVHD) is an uncommon but potentially devastating complication following liver transplantation. Recently, it was shown that use of a human leukocyte antigen (HLA)-homozygous donor leading to one-way HLA matching significantly increases the risk of GVHD after living donor liver transplantation (LDLT). However, the precise impact of HLA matching between donor and recipient on the risk of GVHD is not yet clear. We surveyed instances of fatal GVHD following LDLT in Japan and reviewed all 8 cases in detail, especially with respect to HLA matching. Serological typing showed that 7 of those cases had donor-dominant one-way HLA matching in the 3 loci of HLA-A, -B, and -DR, while one had donor-dominant one-way HLA matching in the 2 loci of HLA-A and -DR and identical alleles in the B locus. However, DNA typing revealed that the latter case had 1-way HLA matching in the 3 loci. Further, we analyzed HLA typing of 906 donor-recipient pairs who underwent LDLT. There were 5 cases with donor-dominant one-way matching in 2 loci and 2 with donor-dominant one-way matching in 1 locus. All of those cases except 1, who died from an unrelated cause, are alive without an obvious presentation of GVHD. In conclusion, our results suggest that the total number of loci with donor-dominant one-way HLA matching is important for determining the risk of fatal GVHD following LDLT, and that DNA typing of HLA alleles is indispensable in some cases to identify the true risk of donor-dominant 1-way HLA matching. *Liver Transpl 12:140–145, 2006.*

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Graft-vs.-host disease (GVHD) is an uncommon but devastating complication following liver transplantation that results from the engraftment of T lymphocytes associated with the liver graft. It is characterized by fever, skin rash, diarrhea, or pancytopenia, which usually occurs 2 to 6 weeks after the procedure.[1-3] One-way matching between a human leukocyte antigen (HLA)-homozygous donor and a haploidentical recipient is a recognized risk factor for GVHD after transplantation.[4,5] Although such a combination of donor and recipient HLA is extremely rare in case of cadaver donor liver transplantation, complete donor-dominant 1-way HLA matching between donor and recipient is a realistic possibility in living donor liver transplantation (LDLT) cases, because most of those donors are genetically related to the respective recipients. Recently, it was shown that use of an HLA-homozygous donor resulting in donor-dominant one-way HLA matching significantly increases the risk of developing GVHD after LDLT, and some have insisted that such donors should be completely excluded.[6,7] However, the precise impact of HLA matching between donor and recipient on the incidence of GVHD has not been clarified. In the present study, we reviewed all reported cases of fatal GVHD after LDLT in Japan and focused on the number of loci with HLA matching between donor and recipient.

PATIENTS AND METHODS

In this retrospective study, we investigated the incidence of fatal GVHD following LDLT in Japan at the time of writing and reviewed all the cases, including

Abbreviations: GVHD, graft-vs.-host disease; HLA, human leukocyte antigen; LDLT, living donor liver transplantation.

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ours, and analyzed the ages of the recipient and donor, donor relation, original disease, initial symptom, Grucksberg stage (peak),[8] onset and course, and HLA type of each recipient and donor. Serologic HLA typing had been performed in all donors and recipients for HLA-A, -B, and -DR according to microlymphotoxicity testing using well-standardized alloantisera.[9,10] A polymerase chain reaction technique was subsequently applied if indicated, using DNA samples of the recipients and donors extracted from peripheral blood lymphocytes or preserved lymphocytes, which were typed for HLA-A, -B, and -DR using a polymerase chain reaction sequence-specific primer[11-13] or restriction fragment length polymorphism method.[14,15] In addition, we collected HLA typing results for 906 donor-recipient pairs who underwent LDLT at Nagoya University Hospital and Kyoto University Hospital from October 1990 to March 2004. The diagnosis of GVHD was based on clinical signs characterized by fever, skin rash, diarrhea, or pancytopenia, and supported by pathologic findings of a skin biopsy consistent with GVHD or the presence of a donor-derived chimerism in peripheral blood.[16]

RESULTS

A total of 8 cases of fatal GVHD after LDLT have been identified in Japan, of whom 2 were infants (Table 1). These patients were transplanted from October 1996 to April 2003. The donor age ranged from 20 to 62 yr old, and recipient age in the 6 adult cases ranged from 37 to 62 yr old. Four of the donors were a son, 3 were mothers, and 1 was a sister of the respective recipients. During the donor selection process, 2 donors with a known potential risk of GVHD were selected because of a lack of alternative candidates. Most of the initial symptoms of GVHD were skin rash with or without fever. The median number of days before appearance of the initial symptom was 40 (range, 14-114), and the median number of days after transplantation to death was 130 (range, 36-540). Regarding peak Gluckberg clinical grade, 4 of the cases were grade 3 and 2 were grade 4.

As for HLA matching between recipient and donor, the serological technique showed that all cases except 1 (case 8) had donor-dominant one-way HLA matching in 3 loci of HLA-A, -B, and -DR, while case 8 had donor-dominant one-way HLA matching in 2 loci of HLA-A and -DR with the B locus neutral because of the homozygote status of the recipient. Following the onset and diagnosis of GVHD, HLA of both the donor and the recipient in the latter case was retyped using the PCR technique, which revealed donor-dominant one-way HLA matching in all 3 loci due to a minor heterogeneity in locus B of the recipient. Consequently, all 8 cases of fatal GVHD after LRLT identified in Japan had donor-dominant one-way HLA matching in 3 loci of HLA-A, -B, and -DR.

Nine cases with donor-dominant one-way matching in 3 loci were identified among 906 donor-recipient pairs who underwent LDLT (Table 2). Among these 9 pairs, fatal GVHD occurred in 4 cases that were included in 8 fatal GVHD cases reviewed in this report. Among the other 5 cases with donor-dominant HLA matching in 3 loci, 3 are alive for a median 61 months without obvious GVHD. Twenty-six cases with donor-dominant 1-way matching in 2 loci were identified among them. Of those, the remaining HLA locus was identical in 5 (both homozygote in 4 and both heterozygote in 1) and 1 haplotype mismatch in 21 (Table 2). There were no cases with donor-dominant one-way matching in 2 loci and 2 mismatch in the other locus. Further, 171 cases with donor-dominant one-way matching in 1 locus were also identified, of which 2 cases were identical in the other 2 loci, 41 were identical in the other 1 locus, and 128 were not identical in the other 2 loci. Five cases with donor-dominant one-way matching in 2 loci and an identical combination in the other locus, and 2 cases with donor-dominant one-way HLA matching in 1 locus and an identical combination in the other 2 loci were identified among the 906 donor-recipient pairs who underwent LDLT. All of these cases were alive at the time of writing without clinical presentation of GVHD except for 2 patients who died (cases 3 and 5), 1 because of an intracerebral hemorrhage and 1 from idiopathic thrombocytopenic purpura and autoimmune hemolytic anemia, of whom neither had clinical signs of skin rash, diarrhea, or fever (Table 3). Although these HLA combinations also seem to contribute theoretically to GVHD, none received the diagnosis of GVHD; this suggests that the number of vectors directing GVHD contribute to the risk of fatal GVHD.

DISCUSSION

GVHD following liver transplantation is difficult to control and devastating in most cases. Clinical symptoms of GVHD after liver transplantation typically become overt between 2 and 8 weeks after transplantation,[1] which is consistent with all cases except 1 in our review. That patient developed GVHD 114 days following transplantation, which was presented first as a skin rash. We could not find any appropriate explanation for the relative delay of the onset in this case. Nemoto et al.[6] reported that case as chronic GVHD after liver transplantation, and it was controlled with administrations of methylprednisolone and tacrolimus, though we later confirmed by personal communication that the patient died from infection due to GVHD. The median onset time of transfusion-associated GVHD is about 10 days[17]; thus, GVHD following liver transplantation can occur late as compared with transfusion-associated GVHD.

As for the donor relationship, in 4 of the 8 cases of fatal GVHD after LDLT the donors were a son, while in 3 they were the mother, and in 1 the sister. This finding demonstrates that GVHD derived from one-way HLA matching can occur even between siblings.

The clinical symptoms of GVHD are generally characterized by fever, skin rash, diarrhea, and/or pancytopenia. Unlike GVHD that follows allogeneic bone marrow or stem cell transplantation, where the biliary epithelium is one of the major targets that results in

TABLE 1. Clinical Characteristics, Donor and Recipient HLA, Initial Symptom, and Clinical Course of the Cases with Fatal GVHD After LDLT

Case no.	Age	HLA			Original disease	Donor relationship	Onset (POD)	Initial symptons	Other symptons	Death (POD)	Glucksberg clinical grade (peak)
		A locus	*B* locus	*DR* (*DRB1**)							
1											
Donor	35	24,–	52,–	*1502,–	PBC	Sister	24	Skin rash	Diarrhea, fever	36	Grade 3
Recipient	37	24,31	52,62	*0901,*1502							
2											
Donor	27	*2402,–	*5201,–	*1502,–	HCC	Son	38	Skin rash, fever	Diarrhea, pancytopenia	149	Grade 3
Recipient	59	*2402,*3101	*5201,*3501	*1502,*0803							
3											
Donor	20	33,–	44,–	6,–	HCC, alcohol	Son	35	Fever, diarrhea	Skin rash, leukopenia	61	Grade 4
Recipient	48	33,24	44,62	6,4							
4											
Donor	31	24,–	52,–	15,–	HCC, HCV	Son	42	Skin rash	Fever	139	Grade 2
Recipient	62	24,31	52,51	15,14							
5											
Donor	32	A3301/03,–	*4402,–	*1301–02,–	PBC	Son	114	Skin rash	Fever	540	Grade 2
Recipient	50	*3301/03, *2601–07	*4402,*4006	*1301–02,*09012							
6											
Donor	30	24,–	52,–	*1502,–	BA	Mother	14	Skin rash, fever	Diarrhea, pancytopenia	43	Grade 4
Recipient	SM	24,2	52,61	*1502,*0802							
7											
Donor	26	2,–	44,–	13,–	BA	Mother	23	Skin rash	Fever, diarrehea	217	Grade 3
Recipient	6M	2,23	44,54	13,04							
8											
Donor	62	*3303,–	44,-(*4403,–)	*1302,–	Alcohol	Mother	24	Skin rash	Fever, diarrhea	36	Grade 3
Recipient	38	*3303,*0301	44,- (*4403,*4402)	*1302,*0403							

Abbreviations: HLA, human leukocyte antigen; LDLT, living donor liver transplantation; PBC, primary biliary cirrhosis; HCC, hepatocellular carcinoma; BA, biliary atresia; POD, postoperative day.

TABLE 2. Donor-Recipient HLA in Cases With Donor-Dominant 1-way Matching

Relation of HLA matching between donor and recipient	Number of cases		GVHD incidence
Donor-dominant 1-way HLA matching at 3 loci	9		4 (44%)
Donor-dominant 1-way HLA matching at 2 loci	26		0
The other locus			
Homo-homo identical		4	
Hetero-hetero identical		1	
1-mismatch		21	
2-mismatch		0	
Donor-dominant 1-way HLA matching at 1 locus	171		0
The other 2 loci			
Identical in both loci		2	
Identical in one locus		41	
Not identical in both loci		128	

TABLE 3. Cases With Donor-Dominant 1-Way Matching at 2 Loci or 1 Locus and Identical at the Other Locus

Case no.	Age	HLA			Original disease	Donor relationship	Outcome (follow-up)
		A locus	*B* locus	*DR* (*DRB1**)			
1							
Donor	34	24,–	62,–	4,–	BA	Father	Alive (13 yr)
Recipient	2	24,–	62,51	4,9			
2							
Donor	33	24,–	61,–	10,–	BA	Father	Alive (8 yr)
Recipient	1	24,–	61,35	10,9			
3							
Donor	42	11,–	39,–	8,–	BA	Father	Death (36 days)
Recipient	9	11,–	39,35	8,4			
4							
Donor	41	24,33	44,–	13,–	BA	Mother	Alive (7 yr)
Recipient	16	24,33	44,52	13,15			
5							
Donor	27	24,– (*2402,–)	7,– (*0702,–)	1,– (*0101,–)	HCC	Son	Death (5 months)
Recipient	57	24,– (*2402,–)	7,52 (*0702,*5201)	1,15 (*0101,*1502)			
6							
Donor	56	33,–	44,–	13,–	FHF	Father	Alive (2 yr)
Recipient	23	33,–	44,–	13,8			
7							
Donor	46	24,–	55,62	4,–	Caroli's disease	Father	Alive (2 yr)
Recipient	16	24,–	55,62	4,9			

Abbreviations: HLA, human leukocyte antigen; LDLT, living donor liver transplantation; BA, biliary atresia; HCC, hepatocellular carcinoma; FHF, fulminant hepatic failure; POD, postoperative day.

abnormal liver function,[5] the transplanted liver is not a target of GVHD, as both the liver and the immunocompetent cells responsible have the same donor origin.[2,16] Taylor et al.[2] noted that the outcome of GVHD is closely related to its clinical pattern, with prognosis particularly poor in those patients who were presented with a fever, as 29 of 30 (97%) reported adult cases died following presentation with fever, while patients with a skin rash alone survived. All of the presented patients with fatal GVHD developed not only a skin rash, but also a fever as the initial symptom or later. Therefore, fever accompanying skin rash seems to be an important prognostic sign in GVHD following LDLT irrespective of whether it is the initial symptom or not. And each was rated as greater than Glucksberg grade 2. Glucksberg et al.[8] reported that there were no significant differences between grades 0 and 1, grades 2 and 3, and grades 3 and 4 for survival, whereas the difference between grades 1 and 2 was highly significant. However, evaluation of the severity of GVHD following liver transplantation based on Glucksberg grade may be difficult, because liver function is usually normal in those patients.

Close HLA matching between donor and recipient is one of the risk factors for the development of GVHD.[1,2] Several authors have reported that use of a graft from an HLA-homozygous donor with 1-way donor-recipient HLA matching led to an extremely high risk of developing GVHD in LDLT.[7,18,19] However, none have analyzed the differential impact of the number of loci with one-way HLA matching on the risk of fatal GVHD following LDLT. Homozygosity at all HLA loci is not as rare as might be expected from a mathematical calculation of all haplotypes.[20] One study found that 1.6% of Caucasian blood donors demonstrate this condition.[21] This is in contrast with the data from the Japanese Red Cross Society showing that approximately 3.2% of blood donors in Japan have the condition. While the probability of one-way HLA matching between nonrelatives is 1 in 800, it is 1 in 100, 1 in 190, and 1 in 180 in combinations of parent-child, sibling-sibling, and grandparent-grandchild, respectively.[22] Therefore, the risk of encountering donor-dominant one-way HLA matching may be extremely high in an LDLT setting as compared to with a cadaver donor.[7] Kiuchi et al.[19] analyzed a large series of LDLT cases and reported that one-way HLA matching in 2 or more loci prone to GVHD was 3.9% (1.4% in 3 loci), and 1 in 4 cases with complete one-way HLA matching in 3 loci died from GVHD. Therefore, the risk of GVHD after liver transplantation seems to be high in Japan, though several cases have also been reported in the United States despite the theoretically low risk.[1,18]

We investigated HLA matching between the recipient and donor in all cases of fatal GVHD after LDLT in Japan. Undiagnosed GVHD may have occurred, because the early symptoms are often nonspecific and often self-limited, making it difficult to distinguish from an infectious disease or drug reaction. DNA typing demonstrated that all of the cases had donor-dominant one-way HLA matching in the 3 loci of HLA-A, -B, and -DR. Fatal GVHD has not occurred in any cases with donor-dominant one-way matching in 2 loci or those with 1-way matching in 1 locus, despite that such 1-way matching theoretically contributes to GVHD, because the host defense system of the recipient is unable to recognize and eliminate donor cells, and donor cells recognize the unshared haplotypes as foreign and react against them.

Our results suggest that the risk of fatal GVHD following LDLT may depend on the number of loci with donor-dominant one-way HLA matching. This is very important information for donor selection and can help avoid unnecessary donor exclusion. However, additional investigations of whether fatal GVHD can occur in cases with donor-dominant one-way matching in 1 or 2 loci must be performed carefully, as well as discussion of cases that did not develop GVHD despite donor-dominant one-way matching in 3 loci.

In conclusion, homozygous donor with one-way donor-dominant HLA matching at 3 loci should be excluded if possible, because of the very high risk of developing fatal GVHD. However, in those with donor-dominant one-way HLA matching at 2 or fewer loci, the evidence for exclusion is not sufficient. Therefore, when such a donor is the only candidate for LDLT, it seems acceptable, following fully informed consent to the theoretical risk of GVHD. In addition, DNA typing of HLA alleles is strongly recommended for combinations carrying a suspected risk of GVHD.

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Intestinal MDR1/ABCB1 level at surgery as a risk factor of acute cellular rejection in living-donor liver transplant patients

Background: Although the prevention of immunologic reactions with sufficient immunosuppression prolongs graft and patient survival rates, the large interindividual variation in tacrolimus pharmacokinetics interferes with treatment. In this study we have examined whether intestinal MDR1 (ABCB1) is a potential biomarker predicting the occurrence of acute cellular rejection, as well as a factor to predict absorption of tacrolimus, after living-donor liver transplantation.

Methods: By use of tissue specimens of intestinal mucosa (n = 164) obtained at surgery, the messenger ribonucleic acid (mRNA) expression of intestinal MDR1 and cytochrome P450 (CYP) 3A4 was quantified.

Results: The probability of acute cellular rejection during the first 10 days after surgery was significantly associated with the average trough concentration of tacrolimus between postoperative days 2 and 4 (45.1% for <7 ng/mL versus 22.9% for >7 ng/mL, $P = .0040$). High levels of MDR1 were associated with an episode of acute cellular rejection before postoperative day 10 (odds ratio, 2.306 [95% confidence interval, 1.058-5.028]) and with a poor survival rate during the first postoperative year (odds ratio, 7.413 [95% confidence interval, 1.567-36.073]). The mRNA expression level of MDR1 was inversely correlated with the tacrolimus concentration–oral dose ratio during the initial 4 days after surgery in patients with a graft-to-recipient weight ratio greater than 1.5 ($r = -0.6798$, $P < .0001$) and those with a graft-to-recipient weight ratio of less than 1.5 ($r = -0.7180$, $P < .0001$).

Conclusion: The enterocyte MDR1 mRNA level was suggested to be a risk factor for acute cellular rejection and death after surgery. Therefore obtaining a sufficient tacrolimus blood level via this molecular information–based initial dosage adjustment may enable the episode of acute cellular rejection after liver transplantation to be reduced. (Clin Pharmacol Ther 2006;79:90–102.)

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Living-donor liver transplantation and subsequent immunosuppressive therapy are well acknowledged to provide excellent results and are usually used in coordination with a cadaveric organ transplant program.[1,2] In countries where cadaveric donors are limited, living-donor liver transplantation is often the only treatment option for patients with end-stage liver disease.[3] Because loss of the graft liver will lead to death, postoperative immunosuppressive therapy is essential to protect the grafted liver from immunologic reactions. As acute cellular rejection occurs mostly within 6 weeks of a transplant,[4] high-dose steroid pulse therapy or anti-CD3 monoclonal antibody treatment is required to save the graft liver.[5,6] Subclinical rejection, where cytologic or histologic signs of rejection exist in the absence of clinical dysfunction of the graft, is particularly frequent (incidence of around 59%) between days 5 and 14 after

liver transplantation.[6] Twenty-five percent of patients with subclinical rejection are treated with high-dose steroid injections. Therefore early exposure to immunosuppressants could reduce the frequency of acute cellular rejection including subclinical rejection. However, these antirejection treatments lead to overimmunosuppression and an infectious state, which are closely associated with death.[7] Although there is a need to protect patients from opportunistic infections including enterobacterium, Epstein-Barr virus, cytomegalovirus, and herpes simplex virus after antirejection treatment, some anti-infectious treatments with antibiotics, antifungal agents, and antiviral drugs are accompanied by drug-induced hepatic and renal dysfunction.[8,9] In addition, high-dose steroid injections should be avoided in patients carrying the hepatitis B or C virus, because steroidal drugs allow the amplification of these viruses in the graft liver and accelerate the recurrence of virus-related hepatitis and cirrhosis.[10,11] Therefore acute cellular rejection should be avoided to prevent further complications, especially immediately after transplantation.

The calcineurin inhibitor tacrolimus (FK-506) has been used as a primary immunosuppressive agent in orthotopic liver transplantation.[12] Therapeutic drug monitoring has facilitated maintaining the blood concentration of tacrolimus within a narrow therapeutic range (between 10 and 20 ng/mL) to prevent side effects such as nephrotoxicity, neurotoxicity, and life-threatening infection.[13,14] However, the bioavailability of orally administered tacrolimus is variable, ranging from 4% to 89% (with a mean value of about 25%),[15,16] and a dosage regimen for the drug immediately after transplantation has yet to be established. This can be attributed to several factors, including poor absorption or extensive first-pass metabolism in the intestine and liver. Therefore a rational dosage regimen for tacrolimus should be determined as early as possible, focusing on its pharmacokinetic interindividual variability.

Tacrolimus is principally metabolized by cytochrome P450 (CYP) 3A subfamilies in the liver. The contribution of active secretion by P-glycoprotein (the product of the *MDR1/ABCB1* gene) and the metabolism by CYP3A expressed in enterocytes are acknowledged as factors influencing the bioavailability of tacrolimus.[17] We reported that the intraindividual variation in the concentration/dose (C/D) ratio of tacrolimus was closely related to the variation in the enterocyte messenger ribonucleic acid (mRNA) expression level of MDR1, but not CYP3A4, in recipients of living-donor small-bowel transplantation.[18,19] Similar results were obtained in patients after living-donor liver transplantation during the initial 7 days after surgery.[20,21] Because of the small number of cases in our past reports, we could not analyze the relationship between the intestinal mRNA expression level of MDR1 and endpoints such as acute cellular rejection. It is necessary to clarify the clinical significance of the intestinal expression level of MDR1 in patients after living-donor liver transplantation to establish the clinical usefulness of adjusting the initial dosage of tacrolimus.

In this study we examined whether the intestinal expression level of MDR1 mRNA could be a molecular marker for acute cellular rejection episodes in patients after living-donor liver transplantation with more enrolled patients, as well as the potential contribution of the molecular information to initial dose setting.

METHODS

Patients and mucosal specimens. The study included 164 patients, having first provided written informed consent, who were enrolled consecutively between November 1998 and December 2004 in whom tissue specimens had been obtained at surgery. The donor was a parent in 119 cases, a spouse in 14, a sibling in 12, an offspring in 12, a grandmother in 3, an uncle in 2, an aunt in 1, and a father-in-law in 1. The demographics of the recipients are listed in Table I. The clinical samples of the upper jejunum were obtained from a part of the Roux-en-Y limb for biliary reconstruction or from a part of the mucosal specimen around the bile drainage tube at living-donor liver transplantation.[22] This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by the Ethics Committee of Kyoto University, Kyoto, Japan; each adult patient and each parent of small children provided written informed consent.

Dosage regimen of tacrolimus, analysis of blood samples, and criteria for acute cellular rejection. The basic immunosuppression regimen consisted of tacrolimus with low-dose steroids.[23] To cover the immediate postoperative period (the day of living-donor liver transplantation, day 0, and postoperative day 1), induction of immunosuppressive therapy was started from the day before the operation, except in cases of hepatic encephalopathy and severe infection. Tacrolimus was administered orally at a dose of 0.075 mg/kg body weight every 12 hours from the evening of day 1.[13,23] The target for the post-transplantation whole-blood trough concentration of tacrolimus was 10 to 12 ng/mL during the first 2 weeks. Steroid treatment was started at graft reperfusion at a dose of 10 mg/kg, with a gradual reduction from 2 $mg \cdot kg^{-1} \cdot d^{-1}$ to 0.3 $mg \cdot kg^{-1} \cdot d^{-1}$ during the first 2 weeks after surgery.

Table I. Demographic characteristics of recipients (N = 164)

Age (y)	0.3-67 (median, 3.2)
Adults (≥15 y) (n = 54)	15.0-67 (median, 46)
Children (<15 years) (n = 110)	0.3-13.7 (median, 1.2)
Body weight (kg)	4.3-92.1 (median, 13.5)
Graft-to-recipient weight ratio (%)	0.63-5.6 (median, 1.84)
Gender (male/female)	70/94
Graft lobe (left/right)	113/51
ABO blood group match (identical/compatible/incompatible)	99/37/28
Preoperative condition (home-bound/hospitalized/intensive care unit–bound)	71/85/8
Primary disease*	
Biliary atresia	89 (21)
Cirrhosis	
Hepatitis B virus	9 (1)
Hepatitis C virus	12 (3)
Primary biliary cirrhosis	7 (2)
Unknown	3 (0)
After liver transplantation	11 (3)
Primary sclerosing cholangitis	8 (4)
Fulminant hepatic failure	4 (2)
Other†	21 (6)

*The number of patients with acute cellular rejection episodes during the initial 10 days after surgery is denoted in parentheses.
†The primary disease was Byler disease in 4 cases (2), Alagille syndrome in 3 (0), Wilson disease in 2 (2), hepatoblastoma in 3 (0), polycystic liver disease in 2 (1), biliary dilation in 2 (0), multiple hepatocellular carcinoma in 1 (0), citrullinemia in 1 (1), hypertyrosinemia in 1 (0), Budd-Chiari syndrome in 1 (0), and portal vein deficiency in 1 (0). The number of patients with acute cellular rejection episodes during the initial 10 days after surgery is denoted in parentheses.

The dosage of tacrolimus was adjusted on the basis of whole-blood trough concentrations measured about 12 hours after the evening dosage every day, by use of a semiautomated microparticle enzyme immunoassay (IMX; Dainabot, Tokyo, Japan).[24]

Acute cellular rejection was principally diagnosed with liver biopsy specimens, and the histologic diagnosis was performed according to criteria based on the Banff schema.[25] All episodes of rejection were treated with a high-dose steroid bolus injection.

Evaluation of intestinal expression levels of MDR1 and CYP3A4. Biopsy specimens from intestinal mucosa were homogenized in RLT buffer (Qiagen, Hilden, Germany), and total RNA was isolated with MagNA-Pure LC RNA Isolation kit II (Roche) and reverse-transcribed as described previously.[26] The isolated total RNA (500 ng/40 μL reaction mixture) was reverse-transcribed by Superscript II reverse transcriptase (Invitrogen, Carlsbad, Calif) with random primers (100 ng/reaction) and digested by RNase H (Invitrogen). After dilution of the single-stranded deoxyribonucleic acid (DNA) mixture with 60 μL of sterile water (final volume, 100 μL), 5-μL aliquots were used for a subsequent real-time polymerase chain reaction (PCR) (final volume, 20 μL) with an ABI PRISM 7700 sequence detector (Applied Biosystems, Foster, Calif). The primer/probe set used for glyceraldehyde 3-phosphate dehydrogenase, as an internal control, was predeveloped TaqMan Assay Reagents (Applied Biosystems), and the reaction was performed according to the manufacturer's instructions. The primer/probe set specific for MDR1 and CYP3A4 was as described previously.[26] Each PCR fragment of the target sequences was generated with specific primer/probe sets as described, ligated into the pCR-Script Cloning Vector (Stratagene, La Jolla, Calif), and confirmed to have the exact sequences of the cloned amplicons by the chain-termination method by use of a fluorescence 373A DNA sequencer (Applied Biosystems). After measurement of the concentrations of the purified plasmid DNA by spectrophotometry, the corresponding concentrations (in moles per microliter) were calculated and serial dilutions of respective plasmid DNA were used as standards for calibration curves. The starting mRNA concentration of MDR1 or CYP3A4 was established by determining the fractional PCR threshold cycle number at which a fluorescence signal generated during the replication process passed above a threshold value. The initial amount of target mRNA in each sample was estimated from the experimental fractional PCR threshold cycle value with a standard curve generated by use of known amounts of standard plasmid DNA.

Statistical analyses. Normally distributed values were presented as mean ± SD. Values that were not

normally distributed were presented as the median and range. Logarithmic transformation of the mRNA levels of MDR1 and CYP3A4 was performed to improve normality before statistical analyses were performed. The nonpaired Student t test was used to compare groups with respect to normally distributed variables. If different variances between 2 samples were found with the F test, an unpaired t test with Welch correction was performed. The Mann-Whitney U test was used to compare groups without normality. The calculated mRNA expression levels of MDR1 and CYP3A4 in each intestinal specimen were categorized as high or low, if the quantified value for the mRNA in question exceeded or fell below the median value for all specimens, respectively. Statistical tests were 2-tailed, and significance was defined as $P < .05$.

The outcome measure studied was immunologic events and survival, defined as the time from living-donor liver transplantation to the first episode of acute cellular rejection during the initial 10 days after surgery and to death during the first year after surgery, respectively. The patients without complications until at least postoperative day 10 were categorized as the event-free group. The patients who were diagnosed with acute cellular rejection by liver biopsy before postoperative day 10 were categorized as the acute cellular rejection group. The probability analysis was performed according to the method of Kaplan and Meier, and the outcome was compared among the subgroups by use of a 2-tailed log-rank test for univariate comparisons. An odds ratio was calculated for the risk. Statistical analyses were performed by use of the statistical software package StatView (version 5.0; Abacus Concepts, Berkeley, Calif).

RESULTS

Patients. Table I shows the demographics and primary diseases of living-donor liver transplant recipients whose mucosal samples we studied. Of the recipients who had acute cellular rejection, 28, 11, and 3 had an ABO blood type that was identical, compatible, and incompatible with that of their donor, respectively. Moreover, 18, 21, and 3 recipients with acute cellular rejection were home-bound, hospitalized, and intensive care unit (ICU)–bound, respectively, before surgery. Of the recipients with acute cellular rejection, 29 and 13 had a graft from the left lobe and right lobe, respectively. Steroid-pulse therapy was used in 32 patients without acute cellular rejection during the first 10 days after surgery, and 11 post–liver transplant patients were treated with immunosuppressants until immediately before the second transplantation. Therefore these 43 patients were excluded from the analyses for the probability of acute cellular rejection but not from the analyses on gene expression and tacrolimus pharmacokinetics, and the analyses for acute cellular rejection were performed with the findings of the other 121 recipients, including 82 event-free patients and 39 acute cellular rejection patients. The survival analysis was performed with these 121 recipients, including 13 patients who died within 1 year after transplantation.

Acute cellular rejection and postoperative tacrolimus trough level. By comparing the daily trough concentration of tacrolimus between the event-free group (n = 82) and the acute cellular rejection group (n = 39), it was found that the trough concentration at postoperative days 3 ($P = .0075$) and 4 ($P = .0022$) was significantly lower in the acute cellular rejection group (Fig 1). These results suggest that the blood level of tacrolimus immediately after living-donor liver transplantation was associated with the occurrence of acute cellular rejection until postoperative day 10. Then, we examined the relationship between the average trough concentration of tacrolimus between postoperative days 2 and 4 and the complications of patients, because the tacrolimus was usually administered to recipients in the ICU during the first 3 days after liver transplantation. At first, we categorized the patients by the average trough concentrations of tacrolimus between postoperative days 2 and 4. Because a low dosage of tacrolimus was administered to patients at risk of infection or renal impairment from the preoperative status to avoid any further deterioration in condition, the categorization was started from 5 ng/mL, which is considered the lower limit of the initial average concentration of tacrolimus. As shown in Fig 2, the frequency of acute cellular rejection tended to be high in patients with relatively lower tacrolimus blood levels, between 5 and 7 ng/mL. The other complications frequently occurred in the patients whose average tacrolimus trough levels were below 5 ng/mL. The frequency of acute cellular rejection compared with the event-free group tended to be lower in the patients whose average tacrolimus trough levels were maintained above 7 ng/mL. Next, we examined the probability of acute cellular rejection in the recipients dividing the average trough concentration of tacrolimus at 7 ng/mL between postoperative day 2 and 4 (Fig 3). Kaplan-Meier analysis demonstrated that the average trough concentration of tacrolimus immediately after living-donor liver transplantation was significantly associated with acute cellular rejection ($P = .0040$). The resultant odds ratio was 2.772 (95% confidence interval [CI], 1.265-6.075) for the patients whose mean trough level of tacrolimus was

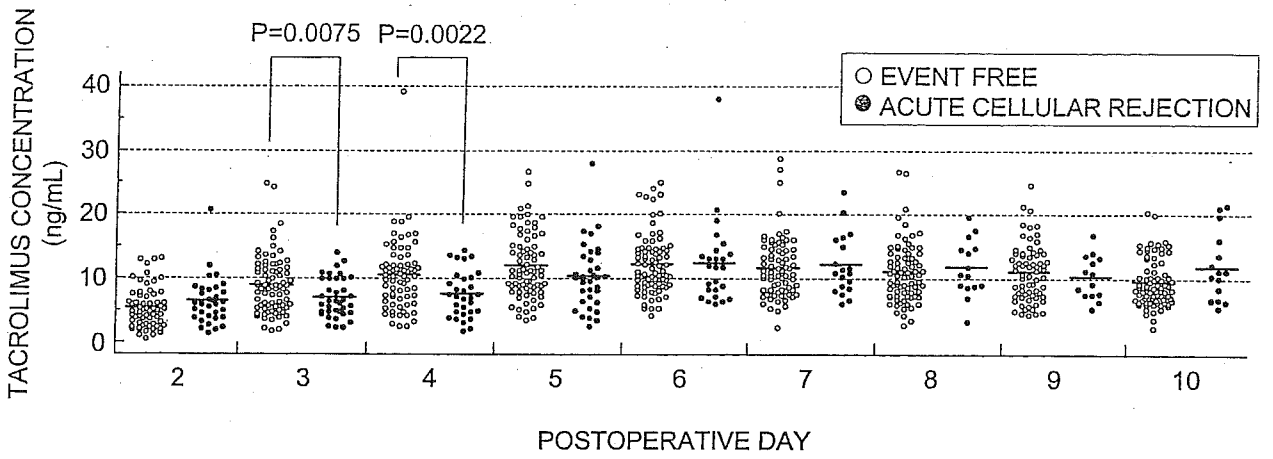

Fig 1. Daily trough levels of tacrolimus in living-donor liver transplant patients. Trough concentrations of tacrolimus in 121 patients receiving de novo living-donor liver transplants are illustrated. The patients are divided into 2 groups: event-free (*open circles*) and acute cellular rejection (*solid circles*). A statistical analysis was performed with the unpaired *t* test after Welch correction. *P* values of less than .05 are shown.

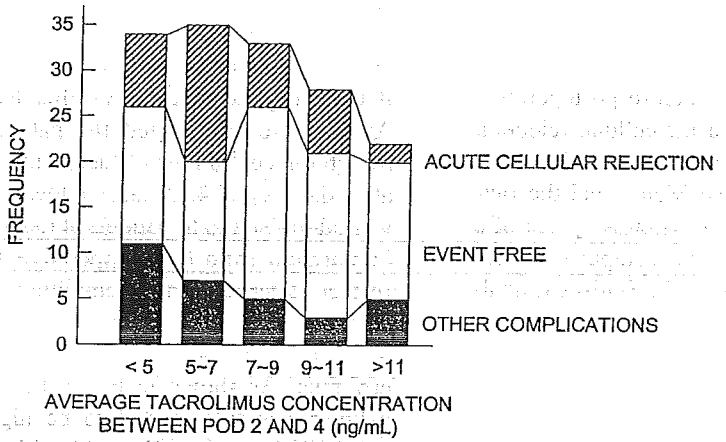

Fig 2. Frequency of complications after living-donor liver transplantation with respect to tacrolimus trough level between postoperative days (POD) 2 and 4. Frequencies of an event-free clinical course, acute cellular rejection, and the need for high-dose steroid treatment for other complications are shown as *open, hatched,* and *solid columns,* respectively. The patients were classified on the basis of the average trough concentration of tacrolimus between postoperative days 2 and 4.

below 7 ng/mL between postoperative days 2 and 4 (Table II).

Association between intestinal mRNA level of MDR1 or CYP3A4 and acute cellular rejection. We previously reported that patients with high levels of enterocyte MDR1, but not CYP3A4, required about 2-fold higher oral dosages of tacrolimus than patients with low levels of MDR1.[20] On the basis of the previous findings, we have re-examined the expression profile of the intestinal mRNA level of MDR1 and CYP3A4 to re-evaluate the influences of these factors on the risk for acute cellular rejection, as well as the interindividual variation of postoperative tacrolimus pharmacokinetics. In Fig 4, *A* and *B*, the logarithmically transformed distribution of the intestinal expression level of MDR1 and CYP3A4 at living-donor liver transplantation is shown. The median value of MDR1 and CYP3A4 was 0.242 amol/μg (range, 0.01-6.51

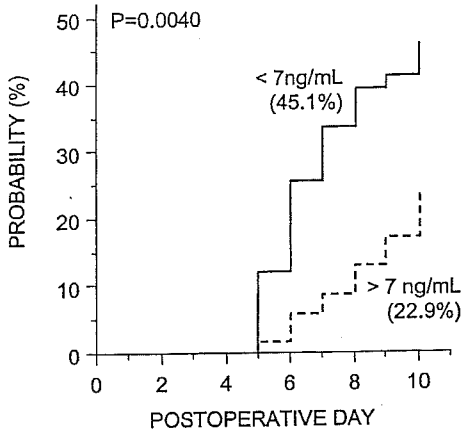

Fig 3. Probability of acute cellular rejection episodes in initial 10 days after living-donor liver transplantation. Kaplan-Meier curves show the probability of acute cellular rejection with respect to the average trough concentration of tacrolimus between postoperative days 2 and 4 (<7 ng/mL or >7 ng/mL). *P* values were determined with the log-rank test.

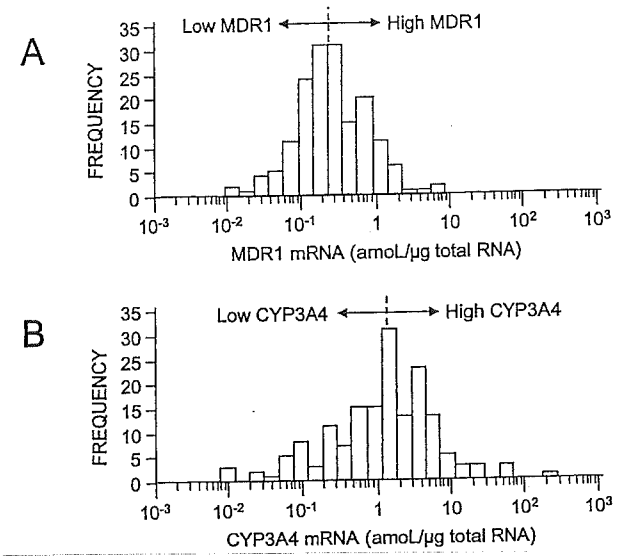

Fig 4. Histograms of messenger ribonucleic acid (mRNA) expression of intestinal MDR1 and CYP3A4 at living-donor liver transplantation. Distribution of mRNA expression levels of MDR1 (**A**) and CYP3A4 (**B**) in intestinal mucosa, both logarithmically transformed to improve normality, are illustrated as histograms for 164 recipients after living-donor liver transplantation. The *dotted lines* denote the median value. RNA, Ribonucleic acid.

amol/µg) of total RNA and 1.278 amol/µg (range, 0.002-185.5 amol/µg) of total RNA, respectively. After dividing the samples by each median value, we examined the probability of acute cellular rejection based on the expression of MDR1 or CYP3A4 (high or low). As illustrated in Fig 5, *A*, a high level of intestinal MDR1 expression was associated with the probability of acute cellular rejection (42.1% in high-MDR1 group versus 23.4% in low-MDR1 group, $P = .0265$). The resultant odds ratio was 2.376 (95% CI, 1.087-5.191) for the patients with a high level of intestinal MDR1 mRNA at living-donor liver transplantation (Table II). However, there was no significant association between the intestinal CYP3A4 mRNA level and the probability of acute cellular rejection ($P = .9211$) (Fig 5, *B*). The odds ratio showed that a high level of CYP3A4 mRNA at liver transplantation was not a risk factor for the occurrence of postoperative acute cellular rejection (Table II). Moreover, the mRNA expression level of mucosal MDR1 in the patients with acute cellular rejection was weakly but significantly higher compared with those in the event-free group ($P = .0476$) (Fig 5, *C*).

Furthermore, the impact of mRNA expression levels of absorptive barriers on patient survival was also examined. According to the method of Kaplan-Meier and subsequent log-rank statistics, the high-level expression of both MDR1 mRNA (Fig 6, *A*) and CYP3A4 (Fig 6, *B*) was significantly associated with patient survival. The odds ratio of the intestinal expression level of MDR1 mRNA at surgery was 7.413 (95% CI, 1.567-36.073), whereas that of CYP3A4 was 3.590 (95% CI, 0.936-13.769).

Table II. Risk factors associated with acute cellular rejection until postoperative day 10

Factors	*Odds ratio*	*95% CI*
Mean trough level of tacrolimus <7 ng/mL between postoperative days 2 and 4	2.772	1.265-6.075
High level of intestinal MDR1 mRNA at surgery	2.376	1.087-5.191
High level of intestinal CYP3A4 mRNA at surgery	1.026	0.485-2.168

CI, Confidence interval; mRNA, messenger ribonucleic acid.

Dosage adjustment based on expression level of intestinal MDR1. To obtain more information about the effect of the intestinal expression level of MDR1 on the pharmacokinetics of tacrolimus, as well as the risk of acute cellular rejection, we compared the daily oral dosage and trough level of tacrolimus between the high- and low-MDR1 groups (Fig 7). The oral dosages