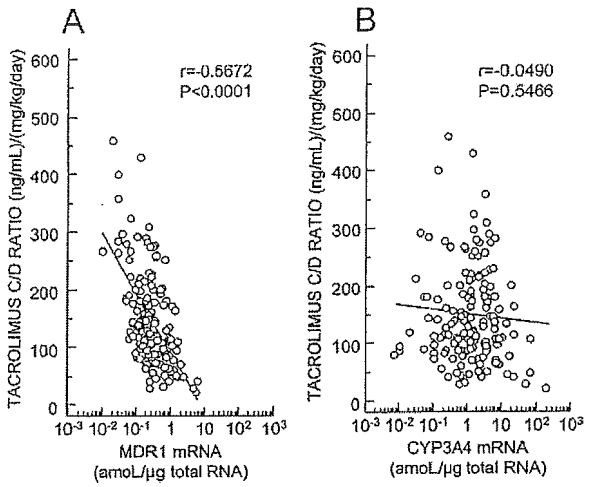

Fig 8. Concentration/dose (C/D) ratio as a function of mRNA expression levels of MDR1 and CYP3A4 in 164 recipients of living-donor liver transplantation. The average C/D ratio in the first 4 days after surgery is compared with the logarithmically transformed mRNA levels of MDR1 (**A**) and CYP3A4 (**B**).

< .0001) for the patients whose graft-to-recipient weight ratio was over 1.5 and those whose ratio was under 1.5, respectively (Fig 9, *B* and *C*). However, the coefficient of the correlation between the intestinal MDR1 mRNA level at surgery and the tacrolimus C/D ratio after postoperative day 5 gradually decreased (Table III).

DISCUSSION

Acute cellular rejection, an early complication of orthotopic liver transplantation, occurs in more than 50% of patients[27-29] and can be diagnosed only by means of a liver biopsy.[25] Although it has minimal impact on either death or late graft function, acute cellular rejection should be avoided to reduce the adverse effects of antirejection treatment. The use of high-dose steroids is the first step in both the induction of immunosuppression and the treatment of acute cellular rejection despite side effects such as osteoporosis, recurrent viral hepatitis, and infections.[30-32] The direct association between acute cellular rejection and patient mortality rate is weak,[7] but this episode would be a trigger for other severe complications such as infections, drug-induced renal injury and neurotoxicity, and recurrence of hepatitis with viral amplification in patients receiving the antirejection treatment. In our study the patients categorized in the high-MDR1 group showed a higher frequency of acute cellular rejection until postoperative day 10 and poor survival within 1 year after surgery (Figs 5 and 6). Therefore the individual patients' clinical history should be explored because "acute cellular rejection" might be hidden behind the diagnosis at death. The incidence of acute cellular rejection in our series was 25.6% (42/164 cases) until postoperative day 10 and 32.2% overall (39/121 cases, excluding 32 patients treated with high-dose steroids for other reasons and 11 cases of post-transplant graft liver failure) (Table I). Of 13 patients who died within 1 year after transplantation, 11 were categorized in the high-MDR1 group. The mortality rate of high-MDR1 patients who had acute cellular rejection early on was 25% (6/24 cases), whereas all 15 patients in the low-MDR1 group were alive despite an episode of acute cellular rejection. In addition, the mortality rate of event-free patients was 15% (5/33 cases) in the high-MDR1 group and 4% (2/49 cases) in the low-MDR1 group. Focusing on the patients with acute cellular rejection during the first 10 days after surgery, the high-level expression of intestinal MDR1 was suggested to be associated with poor survival by χ^2 statistics (6/24 cases in high-MDR1 group versus 0/15 cases in low-MDR1 group, $P = .0352$). Although our results were derived from a relatively small number of cases, the intestinal expression level of MDR1 at surgery could be a prognostic factor in patients with acute cellular rejection at an early phase. If the occurrence of acute cellular rejection can be avoided in the high-MDR1 patients, the mortality rate may be decreased to a level comparable to that in the low-MDR1 group. To reduce the frequency of acute cellular rejection early on, the average trough concentration of tacrolimus during the initial 4 days after surgery should be kept above 7 ng/mL, with an initial dosage adjustment that takes into consideration the intestinal expression level of MDR1 at surgery. In addition, extensive exposure to tacrolimus at an early phase may reduce the mortality rate of patients categorized in the high-MDR1 group.

The intestinal adenosine triphosphate–driven efflux pump MDR1 is considered to play an important role in drug pharmacokinetics.[33] This drug transporter prevents the luminal entry of orally administered drugs such as tacrolimus, cyclosporine (INN, ciclosporin), and sirolimus at apical membranes. Since the report by Hoffmeyer et al,[34] several single-nucleotide polymorphisms (SNPs) in *MDR1* affecting expression or function (or both) have been reported. Notably, C3435T and G2677T/A are detected at a relatively high frequency and have been examined for influences on the drug pharmacokinetics and expression level of the gene product.[34,35] We previously found that these SNPs did

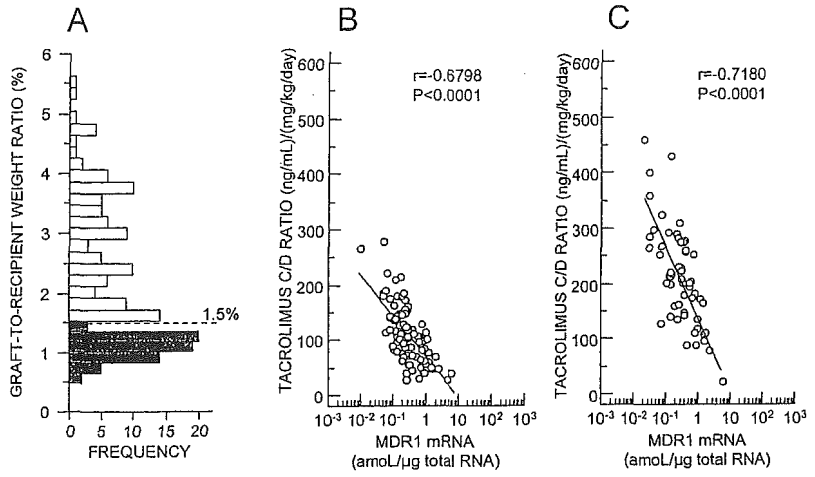

Fig 9. Histogram of graft-to-recipient weight ratio and C/D ratio as a function of mRNA expression levels of MDR1 in 164 recipients of living-donor liver transplantation. A histogram of graft-versus-recipient body weight ratio is shown (**A**). The *dotted line* denotes a graft-to-recipient weight ratio of 1.5. The average C/D ratio in the first 4 days after surgery is compared with the logarithmically transformed mRNA levels of MDR1 in the recipients of living-donor liver transplantation with a graft-to-recipient weight ratio above 1.5 (**B**) and below 1.5 (**C**).

Table III. Coefficient of correlation between intestinal mRNA expression level of MDR1 and tacrolimus concentration/dose ratio in living-donor liver transplantation patients

Postoperative days	*Graft-to-recipient weight ratio >1.5*		*Graft-to-recipient weight ratio <1.5*	
	*r**	*P value*	*r*	*P value*
Between 2 and 4	−0.6798 and (n=99)	< .0001	−0.7180 and (n=55)	< .0001
Between 5 and 7	−0.3721 and (n=90)	.0003	−0.4188 and (n=51)	.0020
Between 8 and 14	−0.1598 and (n=98)	.1163	−0.0241 and (n=52)	.8660

*Data for patients treated with high-dose steroids including the days of administration and 3 days after withdrawal were excluded.

not affect the intestinal expression of *MDR1* and the tacrolimus C/D ratio in living-donor liver transplant recipients.[21,26] In recipients of renal transplantation, the trough concentrations of cyclosporine and tacrolimus were not influenced by the C3435T SNP.[36,37] In addition, examinations in vitro with a vaccinia virus expression system and mammalian expression system using LLC-PK1 cells indicated that the SNPs in *MDR1* do not affect the membrane expression of P-gp or transport activity of drugs such as digoxin and cyclosporine.[38,39] However, there is little consensus concerning the effect on drug pharmacokinetics by SNPs in *MDR1*.[40] Recently, a meta-analysis by Chowbay et al[41] revealed no significant effect of the *MDR1 C3435T* SNP on either the pharmacokinetics of digoxin or the intestinal expression level of P-glycoprotein. In our study preoperative complications such as cholangitis, hyperbilirubinemia, intestinal congestion, pulmonary hypertension, and renal dysfunction may have affected the expression levels of MDR1, and therefore the direct expression level rather than SNPs of the gene would provide more significant information on liver transplant recipients. On the other hand, CYP3A4 is also expressed in the upper intestinal epithelium and mediates the detoxification of these immunosuppressants at the intestinal wall. Therefore MDR1 and CYP3A4 are considered to provide an "absorptive barrier." We previously demonstrated that the intestinal expression level of MDR1, but not of CYP3A4, was inversely correlated with the tacrolimus C/D ratio in small bowel transplant recipients, as well as patients after living-donor liver transplantation.[18-21] In addition, enhanced expression of MDR1 was associated with a reduction in the bioavailability of cyclosporine in an adult case of living-

donor liver transplantation.[42] In our study we have refined the correlation between the intestinal expression level of MDR1 and tacrolimus C/D ratio during the first 4 days after surgery by taking into consideration the graft-to-recipient weight ratio (Figs 8 and 9). The trough concentration of tacrolimus at postoperative days 3 and 4 was significantly lower in the patients categorized in the acute cellular rejection group than in those in the event-free group (Fig 1). In addition, keeping the initial trough concentration of tacrolimus above 7 ng/mL was suggested to reduce the risk of acute cellular rejection (Fig 3). Therefore the initial adjustment of dosage based on the intestinal MDR1 mRNA level may provide sufficient immunosuppression mediated by tacrolimus with a rapid increase in the blood concentration to around the target range (>7 ng/mL) and help to reduce the frequency of acute cellular rejection.

Some risk factors for acute cellular rejection after liver transplantation such as primary disease, Child's classification, and polymorphisms of several cytokines have been postulated.[43,44] These were considered to be congenital factors for patients receiving living-donor liver transplantation, and there is no individualized treatment to reduce the occurrence of acute cellular rejection in patients categorized in the high-risk group. In this study we have found that both the postoperative blood concentration of tacrolimus and the intestinal mRNA level of MDR1 at surgery are significant risk factors for acute cellular rejection early on (Figs 3 and 5 and Table II). However, these risk factors are relatively acquired issues and can be overcome by maintaining the trough concentration of tacrolimus above 7 ng/mL for at least the first 4 days after surgery. In addition, the intestinal expression level of MDR1 at surgery would be a simple pharmacokinetic marker with which to adjust the initial dosage of tacrolimus after living-donor liver transplantation. Therefore quantification of mucosal MDR1 expression may provide for individualization of the dosage regimen of tacrolimus, especially the initial dosage. In this study most of the tacrolimus concentrations were below 10 ng/mL, especially in the acute cellular rejection group (Figs 1 and 7, *B*). To avoid adverse reactions, doctors might be reluctant to raise the dose of tacrolimus in patients with acute cellular rejection. If we can obtain jejunal biopsy specimens for the quantification of mucosal MDR1, the postoperative immunosuppressant dosage regimen could be established before liver transplantation, enabling the tacrolimus trough level to be reached earlier in patients with or without a high level of intestinal MDR1. Therefore a pretherapeutic determination of the intestinal MDR1 mRNA level was suggested to be useful to predict the initial dosage of tacrolimus required in individual patients and thus reduce the frequency of acute cellular rejection immediately after liver transplantation.

In this study we have confirmed that a high expression level of intestinal MDR1 is a prognostic factor for recipients of living-donor liver transplantation (Fig 6, *A*). Although that of CYP3A4 was also associated with poor survival, the odds ratio was not statistically significant. Therefore it was suggested that the intestinal expression level of CYP3A4 was a prognostic factor resulting from some secondary or unknown mechanism. The intestinal expression level of MDR1 was clearly related to the oral clearance of tacrolimus until postoperative day 4 and the occurrence of acute cellular rejection up to postoperative day 10 (Figs 5, *A*, 8, and 9 and Tables II and III). The significant association between the high level of intestinal MDR1 and the 1-year patient survival rate might be explained at least partly by the prognostic significance of early exposure to immunosuppressive therapy after liver transplantation. Therefore medication during ICU care may be critical to survival, as well as the occurrence of acute cellular rejection. The molecular and immunologic mechanism(s) behind these phenomena should be clarified.

The grafted liver mass gradually regenerated after surgery. Fukudo et al[16] demonstrated kinetically that the clearance of orally administered tacrolimus improved or increased (or both) in the postoperative period. In our study the coefficient of the correlation between the intestinal MDR1 level and the C/D ratio of tacrolimus decreased from postoperative day 5 (Table III). This background and our results suggested that hepatic function and the interindividual variation in the rate of graft liver regeneration were associated at least in part with the reduced contribution of the intestinal MDR1 or large intraindividual variation in the pharmacokinetics of tacrolimus after surgery. Surrogate markers relating to the enzymatic activity associated with the interindividual and intraindividual variation in graft liver function after living-donor liver transplantation are needed.

In conclusion, we have advanced our previous finding that the enterocyte mRNA expression level of MDR1 was a simple and useful pharmacokinetic factor for tacrolimus, especially for adjusting the initial dosage in living-donor liver transplant patients. In addition, the average trough concentration of tacrolimus immediately after living-donor liver transplantation should be maintained above 7 ng/mL

for at least 4 days after surgery to prevent acute cellular rejection. Therefore initial dosage adjustment with consideration of the expression level of MDR1 in the small intestine at living-donor liver transplantation may reduce the frequency of acute cellular rejection.

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LIVER TRANSPLANTATION 12:292-300, 2006

ORIGINAL ARTICLE

Cyclosporine Exposure and Calcineurin Phosphatase Activity in Living-Donor Liver Transplant Patients: Twice Daily vs. Once Daily Dosing

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We have compared the pharmacokinetics and pharmacodynamics of cyclosporine between once- and twice-daily dosing regimens in de novo patients of living-donor liver transplantation (LDLT). A total of 14 patients were enrolled in this study, who had received cyclosporine microemulsion (Neoral) twice a day (BID, n = 5) or once daily in the morning (QD, n = 9) after transplantation. On postoperative day (POD) 6, the QD regimen significantly increased cyclosporine exposure; the blood concentration at 2 hours postdose (C_2) and area under the concentration-time curve (AUC) for 4 hours (AUC_{0-4}), compared with the BID regimen. Moreover, the area under the calcineurin (CaN) activity in peripheral blood mononuclear cells time-curve (AUA) for 12 hours (AUA_{0-12}) and 24 hours (AUA_{0-24}) were decreased by approximately 42 and 25% with the QD regimen relative to the BID regimen, respectively. The C_2 level was significantly correlated with the AUC_{0-4} (r^2 = 0.95), which was negatively related to the AUA_{0-12} with a large interindividual variability (r^2 = 0.59). However, a significant correlation was found between the AUA_{0-12} or AUA_{0-24} and CaN activity at trough time points. According to a maximum inhibitory effect attributable to the drug (E_{max}) model, the mean estimates of E_{max} and the C_b value that gives a half-maximal effect (EC_{50}) for CaN inhibition were not significantly different between the 2 groups, respectively. These findings suggest that a once daily morning administration of cyclosporine may improve oral absorption and help to provide an effective CaN inhibition early after LDLT. Furthermore, CaN activity at trough time points would be a single surrogate predictor for the overall CaN activity throughout dosing intervals following cyclosporine administration. *Liver Transpl 12:292-300, 2006.* © 2006 AASLD.

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Living-donor liver transplantation (LDLT) is now acknowledged as a lifesaving therapy for patients with end-stage liver failure. Cyclosporine is a calcineurin (CaN) inhibitor that has been widely used to prevent acute rejections after liver transplantation.[1] Cyclosporine has a narrow therapeutic range and shows large inter- and intraindividual pharmacokinetic variability. Therefore, therapeutic drug monitoring of trough blood concentrations (C_0) has been required to avoid adverse events such as nephrotoxicity and neurotoxicity.[2] However, the C_0 level does not correlate well with the systemic drug exposure and clinical outcomes.[3,4] Since a microemulsion formulation of cyclosporine (Neoral) has been introduced into organ transplantation, a consis-

Abbreviations: LDLT, living-donor liver transplantation; BID, twice a day; QD, once a day; POD, postoperative day; C_b, blood concentration; C_0, trough blood concentration; C_2, blood concentration at 2 hours postdose; CaN, calcineurin; AUC, area under the concentration-time curve; AUA, area under the CaN activity-time curve; PBMC, peripheral blood mononuclear cell; E_{max}, maximum inhibitory effect attributable to the drug; EC_{50} C_b, value that gives a half-maximal effect.

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tent and reliable absorption with low interindividual variability has been achieved,[5] and the absorption profile has enabled us to predict accurately the drug exposure throughout dosing intervals.[6] In addition, blood cyclosporine concentrations at 2 hours postdose (C_2) have been shown to be a good predictor for the absorption profile, which was measured as the area under the concentration-time curve for the first 4 hours postdose.[7] Levy et al.[8] recently reported that a new monitoring strategy based on C_2 levels was superior to traditional C_0 monitoring for liver transplant recipients in reducing the incidence and severity of acute rejections.

Cyclosporine has been orally administered in 2 divided doses every 12 hours in transplant patients. However, some patients have difficulty in achieving therapeutic C_2 levels early after liver transplantation because of delayed and/or poor absorption,[9] probably caused by postoperative paralytic ileus and external biliary drainage. Furthermore, C_0 levels are frequently elevated by a continuous dose escalation as well as by the decreased metabolic function in the grafted liver within the early postoperative period. A single daily administration of the equivalent cumulative dose of cyclosporine may provide an alternative and cost-saving strategy to the use of intravenous cyclosporine, in terms of lowering C_0 levels while ensuring adequate C_2 levels after liver transplantation.[10] Therefore, it is a challenge to improve the pharmacokinetic and pharmacodynamic profiles of cyclosporine by changing the dosing schedule from the traditional twice daily administration to once daily administration in de novo liver transplant patients.

The measurement of CaN phosphatase activity in circulating blood has been considered as a pharmacodynamic approach to evaluate the immunosuppressive effect of cyclosporine.[11] We have recently clarified that the inhibitory effect on CaN activity in peripheral blood mononuclear cells (PBMCs) differed between tacrolimus and cyclosporine in de novo LDLT patients.[12] Tarantino et al.[13] showed no difference in survival and rejection rates in renal transplant recipients given cyclosporine between once-daily and twice-daily dosing groups. However, the optimal protocol or target C_2 levels for once-daily administration of cyclosporine remain to be identified in liver transplant patients. Moreover, no clinical data are available on the temporal CaN inhibition by cyclosporine throughout 24-hour dosing intervals.

The aim of this study was to compare the pharmacokinetics and pharmacodynamics of cyclosporine between twice-daily and once-daily dosing regimens in de novo patients of LDLT. In this study, we measured CaN activity in PBMCs as a pharmacological index of cyclosporine to investigate the relationship between cyclosporine exposure and CaN phosphatase activity.

PATIENTS AND METHODS

Patients and Immunosuppression

This study was performed as a part of the C2-Hour Optimal Use of Neoral in Living Liver Transplantation (CHOPIN) study, which was a pilot study investigating the rational protocol for cyclosporine in LDLT patients managed by C_2 monitoring. We included 14 de novo liver transplant patients in this study. All patients underwent LDLT between September 2003 and June 2005 at the Department of Transplantation and Immunology, Kyoto University Hospital. According to the physicians' decision, cyclosporine was administered twice daily for patients undergoing LDLT before July 2004 (BID group, n = 5), and once daily in the morning for patients receiving LDLT thereafter (QD group, n = 9). The patients' demographics in both groups are summarized in Table 1. The study was conducted in accordance with the Declaration of Helsinki and its amendments, and was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee. Written informed consent was obtained from each patient.

Within 24 hours after LDLT, we started immunosuppression with a microemulsion formulation of cyclosporine (Neoral; Novartis Pharma KK, Tokyo, Japan) and low-dose corticosteroids. For the BID regimen, cyclosporine was orally administered at a fixed dose of 10 mg/kg/day during the first 2 PODs for 2 patients (nos. 1 and 2), and at a fixed dose of 8 mg/kg/day during the first 10 PODs for 3 patients (nos. 3, 4, and 5) (9:00 and 21:00). For the QD regimen, cyclosporine was orally administered at a fixed dose of 8 mg/kg/day during the first 6 PODs (9:00). Since the study was conducted with the intention to treat, the maintenance dose of cyclosporine was adjusted to achieve target blood-cyclosporine concentrations. The target C_2 level was set between 500 and 800 ng/mL for the BID regimen, and between 800 and 1,000 ng/mL for the QD regimen during the first month. When the C_0 level of cyclosporine exceeded 300 ng/mL, the dosage was appropriately reduced. Corticosteroids were administered according to a protocol described previously.[12] Clinical laboratory test markers were measured daily in the morning after transplantation.

Therapeutic Drug Monitoring

Blood samples for the daily C_0 monitoring of cyclosporine were drawn into tubes containing ethylene diamine tetra acetate before the morning dose (8:00) from POD 2. We routinely monitored C_2 levels of cyclosporine in the morning. To evaluate the absorption profile, blood samples were taken before and at 2, 4, 6, and 12 hours after the morning administration on POD 6 and 27 in the BID group, and at the same time points including 24 hours postdose on POD 6 and 13 in the QD group. The concentration of cyclosporine in whole blood was measured with a fluorescence polarization immunoassay method using a TDx analyzer (Abbott Japan, Tokyo, Japan). All samples were assayed on the day of blood collection.

Measurement of CaN Phosphatase Activity

On the day of transplantation, we took a blood sample to determine the baseline CaN activity before cyclospor-

TABLE 1. Demographics of LDLT Patients in BID and QD Groups

Patient	Primary disease	Gender	Age (years)	Body weight (kg)	GRWR (%)	ABO matching
BID group						
1	HCV infection	Male	56	63.0	0.72	Identical
2	HBV infection	Male	62	69.4	1.02	Identical
3	Primary biliary cirrhosis	Female	50	53.9	0.96	Identical
4	HCV infection	Female	53	51.0	0.93	Compatible
5	Biliary atresia	Male	19	51.7	0.85	Identical
Total (n = 5)			48 ± 17	57.8 ± 8.1	0.89 ± 0.11	
QD group						
6	HBV infection	Male	55	66.1	1.04	Identical
7	HBV infection	Male	48	73.0	1.03	Identical
8	HBV infection	Female	30	44.0	1.04	Compatible
9	Primary biliary cirrhosis	Female	56	60.0	1.54	Identical
10	Biliary atresia	Male	19	62.8	0.96	Identical
11	Cirrhosis: unknown causes	Male	52	84.8	0.98	Identical
12	Primary biliary cirrhosis	Female	51	56.0	1.15	Identical
13	Primary biliary cirrhosis	Female	67	60.7	1.03	Identical
14	HBV infection	Male	62	76.0	1.10	Identical
Total (n = 9)			49 ± 15	64.8 ± 12.0	1.10 ± 0.18*	

NOTE: Values expressed as number or mean ± SD.
Abbreviations: LDLT, living-donor liver transplantation; BID, twice a day; QD, once a day; HCV, hepatitis C virus; HBV, hepatitis B virus; GRWR, graft-to-recipient weight ratio; SD, standard deviation.
*Significantly different from the mean value in the BID group ($P < 0.05$).

ine administration in each patient. After the administration, CaN activity was simultaneously measured with the therapeutic drug monitoring of cyclosporine daily during the first 2 weeks of transplantation and on POD 27. Blood samples (approximately 2 mL) were diluted with the same volume of phosphate-buffered saline to isolate PBMCs using Ficoll-Paque Plus (Amersham Biosciences, Uppsala, Sweden) as described previously.[12] The measurement of CaN phosphatase activity in PBMCs was carried out using [γ-^{32}P] RII phosphopeptide, consisting of 19 amino acids (DLDVPIPGRFDRRVSVAAE), as a substrate according to a procedure described previously.[12]

Pharmacokinetic and Pharmacodynamic Analysis

The area under the concentration-time curve for the first 4 hours (AUC_{0-4}), 12 hours (AUC_{0-12}), and 24 hours (AUC_{0-24}) after cyclosporine administration was calculated according to the trapezoidal rule. The highest observed concentration and associated time point were defined as the maximum concentration (C_{max}) and corresponding time (T_{max}), respectively. The apparent clearance (CL/F) in the BID and QD groups was calculated by dividing the morning dose on each study day with the AUC_{0-12} and AUC_{0-24} values, respectively. The area under the CaN activity-time curve over 12 hours (AUA_{0-12}) and 24 hours (AUA_{0-24}) after the administration was calculated according to the trapezoidal rule. Assuming that the BID regimen provided a similar cyclosporine exposure and CaN inhibition during the 2 periods of daily dosing, the values of AUC_{0-24} and AUA_{0-24} were calculated as double the corresponding measurements from 0 to 12 hours, respectively. We also calculated the percentage of inhibition of CaN activity relative to the predose level to evaluate the inhibitory effect of cyclosporine throughout dosing intervals. The greatest observed CaN inhibition, causing a nadir of enzyme activity, and associated time point were defined as the maximum CaN inhibition (I_{max}) and corresponding time (T_{nadir}), respectively.

The relationship between the blood cyclosporine concentration and CaN phosphatase activity in PBMCs was analyzed with the nonlinear mixed effect modeling program NONMEM,[14] using the following maximum effect (E_{max}) model described previously:[12]

$$CaN = CaN_{base} - (E_{max} \cdot C_b)/(EC_{50} + C_b),$$

where CaN is the CaN activity at blood concentration C_b, CaN_{base} is the baseline activity measured on the day of transplantation before drug administration, E_{max} is the maximum inhibitory effect attributable to the drug, and EC_{50} is the C_b value that gives a half-maximal effect. The individual Bayesian post hoc estimates for E_{max} and EC_{50} were calculated using all data from each patient with population pharmacodynamic parameters of cyclosporine derived previously.[12]

Safety was also evaluated based on nephrotoxicity, which was defined as an initial increase in serum creatinine of ≥0.5 mg/dL above the baseline.

STATISTICAL ANALYSIS

Data were presented as the mean ± standard deviation. Statistical analyses were performed using the statisti-

cal software package StatView, version 5.0 (Abacus Concepts, Berkeley, CA). The significance of differences in mean values between the 2 groups was analyzed with the unpaired *t*-test or the paired *t*-test. Correlations between variables were assessed by univariate linear regression analysis. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Pharmacokinetic and Pharmacodynamic Profiles of Cyclosporine

The demographics of patients at LDLT were similar in the BID and QD groups except for the graft-to-recipient weight ratio (Table 1). A total of 344 measurements of the blood cyclosporine concentration and 351 measurements of CaN activity from the 2 groups were examined in this study. Figure 1 shows the daily oral dose of cyclosporine in the 2 groups, and Figure 2 shows the profiles of the blood cyclosporine concentration and CaN phosphatase activity during the first 2 weeks after LDLT. The daily dosage did not differ significantly between the BID and QD groups (Fig. 1). The target C_2 level in the BID group (500–800 ng/mL) was achieved by POD 4, although the C_0 level was remarkably elevated to near 600 ng/mL (Fig. 2). Since the cyclosporine dosage in the BID group was reduced thereafter, the C_2 level failed to reach significantly higher than the C_0 level on POD 6, and became subtherapeutic after POD 10 (Figs. 1 and 2). In contrast, the C_2 levels in the QD group were significantly higher than the C_0 levels, and were maintained well within the target range (800–1,000 ng/mL) without C_0 levels rising above 300 ng/mL throughout the first 2 weeks after LDLT (Fig. 2).

CaN activity relative to the baseline in the BID group did not significantly differ between before dosing and at 2 hours postdose during the first 6 PODs, except on POD 5 (Fig. 2). Although the CaN activity at 2 hours postdose in the BID group was suppressed to around 20 to 50% of the baseline after the first week, the predose activity showed a progressive rising tendency, and became higher than 50% of the baseline after POD 11 (Fig. 2). On the other hand, the CaN activity before dosing in the QD group remained lower than half of the baseline on each POD, excluding POD 2 (Fig. 2). Furthermore, the activity at 2 hours postdose was significantly inhibited compared with the predose activity except on POD 9, and was persistently suppressed to lower than 20% of the baseline throughout the first 2 weeks after POD 2 (Fig. 2).

Cyclosporine-related nephrotoxicity occurred in 4 of 5 patients in the BID group, while only 2 of 9 patients in the QD group experienced the adverse event during the first 2 weeks of transplantation. Within the first few PODs, the mean serum creatinine level in both groups

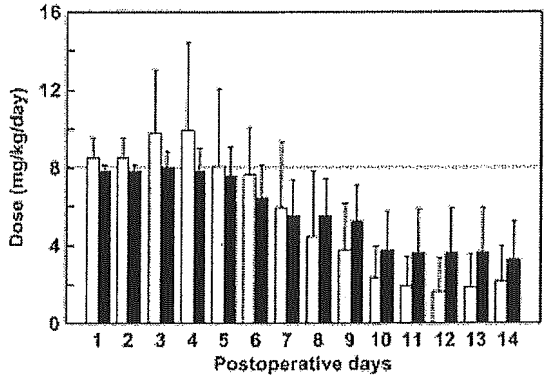

Figure 1. Daily oral dose of cyclosporine in the BID (open columns) and QD (closed columns) groups after LDLT. The dotted line shows the average starting dose for all patients (8 mg/kg/day, n = 14). Each column represents the mean ± standard deviation.

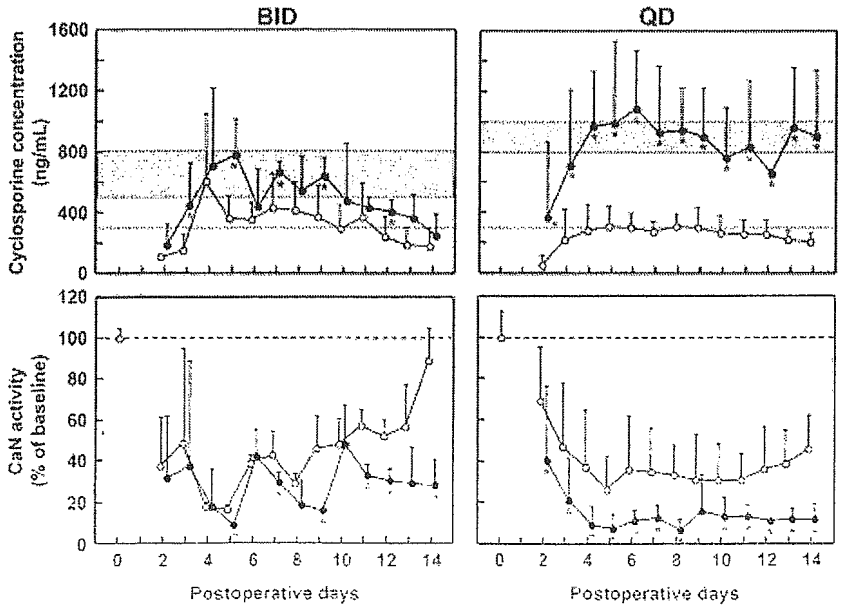

Figure 2. Blood cyclosporine concentration and CaN phosphatase activity in PBMCs in the BID and QD groups after LDLT. Open and closed circles show data measured before dosing and at 2 hours postdose, respectively. The closed areas show the target C_2 level (BID, 500–800 ng/mL; QD, 800–1,000 ng/mL). The dotted lines indicate the upper limit of the C_0 level (300 ng/mL). The dashed lines indicate the baseline CaN activity. Each symbol represents the mean ± standard deviation. *P < 0.05, significantly different from data measured before dosing on the same POD.

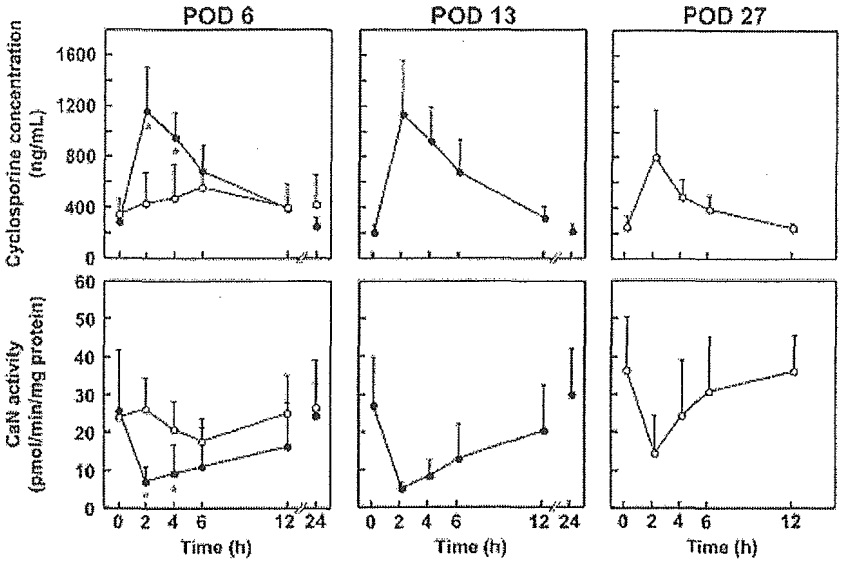

Figure 3. Time courses of blood cyclosporine concentration and CaN phosphatase activity in PBMCs in the BID (open circles) and QD (closed circles) groups on POD 6, 13, and 27. Each symbol represents the mean ± standard deviation. *$P < 0.05$, significantly different from data at the same time point postdose in the BID group.

showed no remarkable change. However, the mean serum creatinine level in the BID group steadily increased after POD 5, and remained above 1.0 mg/dL until POD 14. On the other hand, the mean serum creatinine level in the QD group was overall lower than that in the BID group, and no increase over 1.0 mg/dL was observed throughout the first 2 weeks after transplantation.

Time Course of Blood Cyclosporine Concentration and CaN Phosphatase Activity

Figure 3 shows the temporal profiles of the blood cyclosporine concentration and CaN phosphatase activity on PODs 6, 13, and 27. On POD 6, the C_2 level was almost similar to the C_0 level, and the T_{max} was delayed behind the first 4 hours postdose in the BID group (Fig. 3). In addition, CaN activity throughout dosing intervals in the BID group was only partially inhibited relative to the activity before dosing (Fig. 3). In contrast, the QD regimen using a higher dose per administration improved cyclosporine absorption, and significantly increased the pharmacokinetic parameters of C_2, C_{max}, AUC_{0-4}, and AUC_{0-12} without elevating the C_0 level, compared with the BID regimen (Fig. 3; Table 2). Moreover, the T_{max} became significantly shorter in the QD group than the BID group (T_{max} = 2.8 vs. 6.4 hours; Table 2). The CL/F was smaller in the QD group than the BID group, although the difference was not significant (CL/F = 0.54 vs. 0.96 L/hour/kg; Table 2). Notably, the CaN activity at 2 hours postdose (CaN_2) was significantly decreased, and a more profound maximum CaN inhibition was produced by the QD regimen compared with the BID regimen (I_{max} = 76.0 vs. 32.0%; Table 2). The AUA_{0-12} and AUA_{0-24} were decreased by approximately 42 and 25% with the QD regimen relative to the BID regimen, respectively (Table 2). However, no significant difference was found in the total 24-hour CaN activity (AUA_{0-24}) as well as the corresponding cyclosporine exposure (AUC_{0-24}) between the 2 dosing regimens using a similar daily dose of cyclosporine (Table 2).

The C_2 levels reached around the respective target ranges on POD 13 in the QD group and on POD 27 in the BID group (Fig. 3; Table 2). A maximum CaN inhibition occurred at around 2 hours postdose parallel to the maximal blood concentration on POD 13 and 27 (Fig. 3; Table 2). Moreover, the mean CaN activity recovered to the predose levels by 12 and 24 hours postdose in the BID and QD groups, respectively. While the daily dose of cyclosporine was tapered off on both PODs, comparable AUC_{0-24} and AUA_{0-24} were achieved in the 2 groups to those on POD 6, respectively (Table 2). Notably, the dose-corrected AUC_{0-4} similarly increased in all patients except 2, according to the increase of POD during the first month after LDLT (Fig. 4).

Exposure and Response Relationship of Cyclosporine

The AUC_{0-4} was significantly correlated with the C_2 level ($r^2 = 0.95$; $P < 0.0001$; Fig. 5A), but not with the C_0 level ($r^2 = 0.01$; $P = 0.75$). The correlation of the AUA_{0-12} with AUC_{0-4} did not reach a level of significance because of the large interindividual variability. However, there was a negative trend toward a lower AUA_{0-12} at a higher AUC_{0-4} when the mean values for each group were compared ($r^2 = 0.59$; $P = 0.23$; Fig. 5B). Similarly, no significant correlation was documented between the AUA_{0-24} and AUC_{0-24} ($r^2 = 0.13$; $P = 0.096$; Fig. 5C). On the other hand, a significant correlation was found between the AUA_{0-12} and CaN activity before dosing (CaN_0; $r^2 = 0.58$; $P < 0.0001$) or CaN activity at 12 hours postdose (CaN_{12}; $r^2 = 0.90$; $P < 0.0001$). Furthermore, the AUA_{0-24} was related well with CaN_0 activity ($r^2 = 0.74$; $P < 0.0001$; Fig. 5D) and CaN activity at 24 hours postdose (CaN_{24}; $r^2 = 0.87$; $P < 0.0001$).

TABLE 2. Pharmacokinetic (PK) and Pharmacodynamic (PD) Parameters of Cyclosporine in LDLT Patients

Parameter	POD 6		POD 13	POD 27
	BID (n = 5)	QD (n = 8)	QD (n = 6)	BID (n = 3)
PK parameter				
C_0 (ng/mL)	348 ± 115	282 ± 75	203 ± 59	254 ± 86
C_2 (ng/mL)	434 ± 241	1,160 ± 340*	1,140 ± 424	807 ± 371
C_{max} (ng/mL)	567 ± 294	1,210 ± 286*	1,250 ± 343	807 ± 371
T_{max} (h)	6.4 ± 3.6	2.8 ± 1.0*	2.7 ± 1.0	2.0 ± 0.0
AUC_{0-4} (ng · h/mL)	1,690 ± 821	3,550 ± 789*	3,400 ± 875	2,360 ± 950
AUC_{0-12} (ng · h/mL)	5,610 ± 2,770	8,460 ± 1,600*	8,000 ± 1,830	5,150 ± 1,530
AUC_{0-24} (ng · h/mL)	11,200 ± 5,530†	12,400 ± 2,300	11,200 ± 2,280	10,300 ± 3,060†
Morning dose (mg/kg)	4.2 ± 0.6	6.5 ± 1.8*	4.9 ± 1.5	2.8 ± 0.9
Daily dose (mg/kg/day)	7.6 ± 2.4	6.5 ± 1.8	4.9 ± 1.5	4.1 ± 0.5
CL/F (L/h/kg)	0.96 ± 0.62	0.54 ± 0.14	0.43 ± 0.08	0.62 ± 0.40
PD parameter				
CaN_0 (pmol/min/mg protein)	23.9 ± 1.9	25.8 ± 15.9	26.9 ± 12.6	36.2 ± 14.4
CaN_2 (pmol/min/mg protein)	26.0 ± 8.3	7.2 ± 3.8*	4.8 ± 1.4	14.4 ± 10.1
I_{max} (% inhibition)	32.0 ± 20.8	76.0 ± 9.9*	82.4 ± 10.8	64.1 ± 15.2
T_{nadir} (h)	8.0 ± 3.7	3.3 ± 1.5*	2.7 ± 1.0	2.0 ± 0.0
AUA_{0-12} (pmol · h/min/mg protein)	262 ± 70	152 ± 110*	161 ± 88	345 ± 147
AUA_{0-24} (pmol · h/min/mg protein)	525 ± 140†	395 ± 266	459 ± 224	689 ± 295†

NOTE: Values expressed as mean ± SD.
Abbreviations: LDLT, living-donor liver transplantation; POD, postoperative days; BID, twice a day; QD, once a day; C_0, trough blood concentration; C_2, blood concentration at 2 hours postdose; C_{max}, maximum blood concentration; T_{max}, time corresponding to C_{max}; AUC_{0-4}, area under the concentration-time curve from 0 to 4 hours; AUC_{0-12}, area under the concentration-time curve from 0 to 12 hours; AUC_{0-24}, area under the concentration-time curve from 0 to 24 hours; CL/F, apparent clearance; CaN_0, calcineurin activity before dosing; CaN_2, calcineurin activity at 2 hours postdose; I_{max}, maximum calcineurin inhibition; T_{nadir}, time corresponding to I_{max}; AUA_{0-12}, area under the calcineurin activity-time curve from 0 to 12 hours; AUA_{0-24}, area under the calcineurin activity-time curve from 0 to 24 hours; SD, standard deviation.
*Significantly different from the mean value in the BID group on POD 6 ($P < 0.05$).
†The values were calculated as double the corresponding measurements for 12 hours.

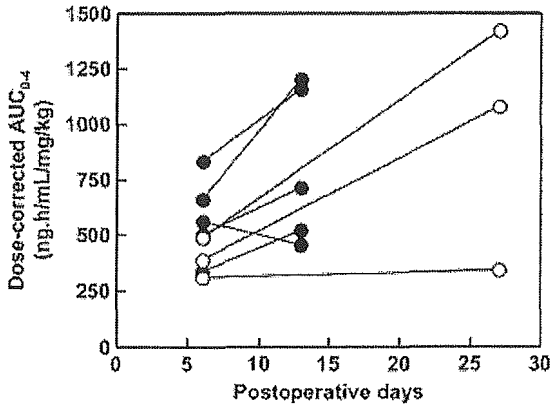

Figure 4. Time-dependent cyclosporine absorption during the first month after LDLT. Open circles represent the dose-corrected AUC_{0-4} from 3 patients in the BID group, for whom data were obtained on POD 6 and 27. Closed circles represent the dose-corrected AUC_{0-4} from 5 patients in the QD group, for whom data were obtained on POD 6 and 13. Data from the same patient are connected.

The relationship between the blood cyclosporine concentration and CaN phosphatase activity in PBMCs well fitted to the E_{max} model in each patient. The results for 3 patients are shown in Figure 6. The average values of individual Bayesian estimates for E_{max} and EC_{50} were not significantly different between the BID and QD groups (Table 3). The interindividual variability for E_{max} was relatively small (% coefficient of variation = 13.3%; Table 3), while the EC_{50} value showed a large variability among the patient population (% coefficient of variation = 65.3%; Table 3). CaN activity was inhibited concentration-dependently by cyclosporine, and the inhibition was almost complete at blood cyclosporine concentrations above 700 ng/mL, which was approximately 4 times the mean EC_{50} value (Fig. 6; Table 3).

DISCUSSION

Since the introduction of the microemulsion formulation of cyclosporine (Neoral), the poor and variable absorption associated with the previous oily formulation has improved.[5] However, it is often difficult to achieve therapeutic C_2 levels in some patients who are given cyclosporine in 2 divided doses immediately after liver transplantation.[9] Oral administration of cyclosporine once a day may potentially overcome this problem by increasing peak blood concentrations without elevating C_0 levels. However, there have been few clinical investigations on the optimal use of cyclosporine as a single daily administration in liver transplant patients. In the present study, we have first compared the pharmacokinetics and pharmacodynamics of cyclosporine in de

novo LDLT patients treated with the BID regimen vs. QD regimen.

On POD 6, the QD regimen significantly improved cyclosporine absorption, and provided comparable 24-hour drug exposure and CaN activity to the BID regimen (Fig. 3; Table 2). During the first week of transplantation, the C_0 levels in the BID group markedly exceeded 300 ng/mL (Fig. 2), which might have been responsible for the subsequent increase in serum creatinine levels and more frequent nephrotoxicity than in the QD group. Furthermore, the C_2 levels in the BID group became subtherapeutic after POD 10 due to the continuous dosage reduction because of the onset of nephrotoxicity (Figs. 1 and 2). On the other hand, the C_2 levels in the QD group were well controlled overall within the target range (800–1,000 ng/mL) without C_0 levels rising above 300 ng/mL, and the CaN activities both before dosing and at 2 hours postdose were persistently suppressed to lower than half of the baseline throughout the first 2 weeks after LDLT (Fig. 2). These preliminary results suggest that the QD regimen may improve the cyclosporine pharmacokinetics and reduce the incidence of nephrotoxicity, without compromising the pharmacodynamic profile for CaN inhibition early after LDLT.

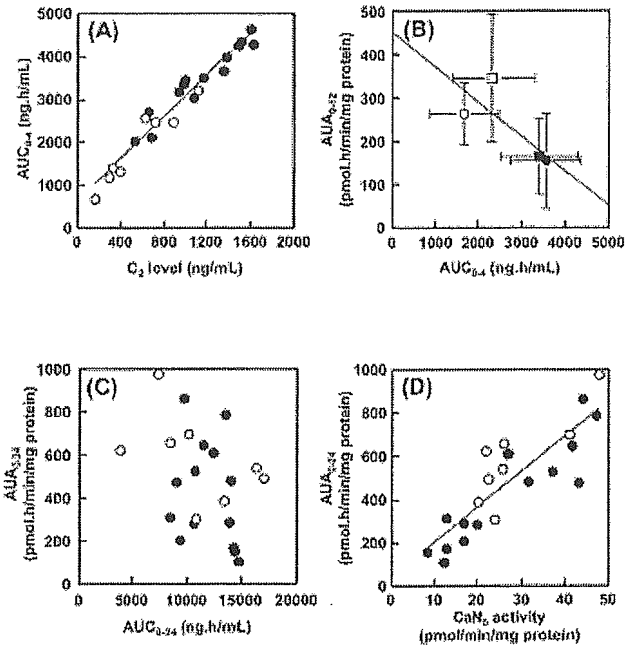

Figure 5. Correlations between pharmacokinetic parameters (A), between exposure and response (B and C), and between pharmacodynamic parameters (D) of cyclosporine in LDLT patients. (A) AUC_{0-4} vs. C_2 level for all points in the BID (open circles) and QD (closed circles) groups. (B) Mean AUA_{0-12} values on POD 6 (open circle) and 27 (open square) in the BID group, and on POD 6 (closed circle) and 13 (closed square) in the QD group were compared with mean AUC_{0-4}, respectively. Error bar indicates standard deviation. (C) AUA_{0-24} vs. AUC_{0-24} for all points in the BID (open circles) and QD (closed circles) groups. (D) AUA_{0-24} vs. CaN_0 activity for all points in the BID (open circles) and QD (closed circles) groups.

Interestingly, the QD regimen using a higher dose per administration significantly increased the rate and degree of cyclosporine absorption compared with the BID regimen on POD 6 (Fig. 3; Table 2). The apparent clearance of cyclosporine in the QD group showed a tendency to decline as compared with that in the BID group on POD 6 (Table 2), suggesting nonlinear changes in the cyclosporine pharmacokinetics. Lown et al.[15] reported that intestinal P-glycoprotein content significantly correlated with the oral bioavailability of cyclosporine. We have recently found that P-glycoprotein plays an important role in limiting the oral absorption of cyclosporine in LDLT patients.[16] Therefore, the nonlinear changes in the dose-concentration relationship might be partly explained by an altered absorption profile caused by the overcoming of the absorptive barrier of P-glycoprotein in the intestine.

A stable absorption of cyclosporine and CaN inhibition parallel to the blood drug concentration were observed on POD 13 in the QD group as well as on POD 27 in the BID group (Fig. 3). Notably, despite a similar 12-hour drug exposure (AUC_{0-12}), the higher C_2 level of cyclosporine produced a greater maximum CaN inhibition (I_{max}) on POD 27 than did cyclosporine on POD 6 in the BID group (Fig. 3; Table 2), indicating that the C_2 level of cyclosporine may be relevant to temporal CaN inhibition. We found that the dose-corrected AUC_{0-4} increased with POD during the first month after LDLT (Fig. 4). Similar results were also reported among de novo liver transplant recipients in the Liver Investigational Study of Neoral C2 vs. Tacrolimus (LIS2T) study,[17] demonstrating a pronounced increase in C_2

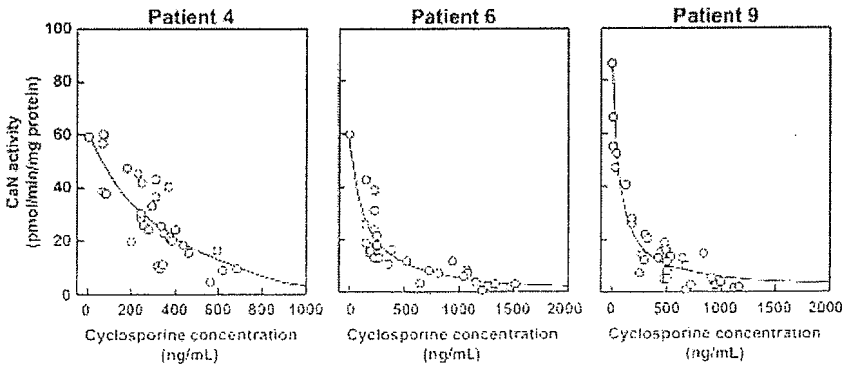

Figure 6. Relationship between blood cyclosporine concentration and CaN phosphatase activity in PBMCs in 3 typical patients of LDLT. Each line shows the predicted CaN phosphatase activity vs. the blood cyclosporine concentration profile using the individual pharmacodynamic parameter estimates (shown in Table 3) according to the E_{max} model.

TABLE 3. Individual Bayesian Estimates for Pharmacodynamic Parameters of Cyclosporine in LDLT Patients

Patient	E_{max} (pmol/min/mg protein)	EC_{50} (ng/mL)
BID group		
1	62.0	199
2	53.1	181
3	61.6	209
4	82.6	465
5	66.3	100
Total (n = 5)	65.1 ± 10.9	231 ± 138
QD group		
6	62.0	132
7	64.9	119
8	70.2	110
9	86.0	64
10	66.7	286
11	67.9	101
12	66.3	302
13	69.3	115
14	56.0	47
Total (n = 9)	67.7 ± 8.1	142 ± 91
All (n = 14)	66.8 ± 8.9 (13.3%)*	174 ± 113 (65.3%)*

NOTE: Values expressed as number or mean ± SD.
Abbreviations: LDLT, living-donor liver transplantation; BID, twice a day; QD, once a day; E_{max}, maximum inhibitory effect; EC_{50}, blood concentration that gives a half-maximal effect; SD, standard deviation.
*The values in parentheses denote % coefficient of variation.

per dose ratio during the first 3 months after transplantation. Although drug metabolism in the grafted liver has been shown to increase according to the increase of POD in LDLT patients given tacrolimus,[18] the apparent clearance of cyclosporine paradoxically decreased over time in both groups (Table 2). These findings imply that the recovery in cyclosporine absorption might be greater than that in the hepatic clearance of cyclosporine within the early postoperative period of LDLT.

As has been shown in a previous study,[7] the C_2 level was significantly correlated with AUC_{0-4} (Fig. 5A). In addition, a negative, but not significant, relationship was observed between the AUC_{0-4} and AUA_{0-12} (Fig. 5B). Caruso et al.[19] reported no significant correlation of the AUC_{0-12} with the area under the CaN activity in whole blood-time curve over 12 hours in renal transplant recipients treated with cyclosporine twice daily. We also found no significant correlation between the AUC_{0-24} and AUA_{0-24} in our LDLT patients (Fig. 5C). However, the AUA_{0-12} and AUA_{0-24} were significantly correlated with the CaN activity at trough time points (Fig. 5D). Therefore, the present findings suggest that C_2 levels may be predictive of cyclosporine absorption as well as CaN inhibition in PBMCs early after LDLT. Furthermore, since a large interindividual variability was found in the relationship between cyclosporine exposure and CaN activity (Figs. 5 and 6; Table 3), the monitoring of CaN activity at trough time points would be useful to predict the overall CaN activity in LDLT patients given cyclosporine once or twice daily.

Hardinger et al.[20] have shown that tacrolimus given once daily at 85% of the twice-daily dose provides safe and equivalent drug exposure to twice-daily dosing in kidney transplant recipients. However, the optimal dosage adjustment or target C_2 levels for the QD regimen of cyclosporine in LDLT patients remain to be clarified. Moreover, it is currently unknown whether the high C_2 levels or AUC_{0-4} achieved by the QD regimen increases the incidence of neurotoxicity such as headache and nausea, although these averse events have been reported to occur within 2 hours postdose in renal transplant recipients given cyclosporine once daily.[21] In the present study, we confirmed that CaN activity was closely related with the blood cyclosporine concentration as reported previously,[12,22] and found that the enzyme activity was almost completely inhibited at blood cyclosporine concentrations above 700 ng/mL (Fig. 6). Taking these findings into consideration, single daily administration of cyclosporine to ensure C_2 levels of around 700 ng/mL without elevating C_0 levels above 300 ng/mL might be safe and effective for adequate CaN inhibition early after LDLT. However, our small sample size does not allow us to draw any conclusions on the clinical usefulness of the once daily dosing regimen of cyclosporine. Further prospective analysis in a large population should be performed to clarify the long-term safety and efficacy of a single daily administration of cyclosporine in de novo LDLT patients.

In conclusion, we have shown that C_2 monitoring is useful to evaluate the absorption profile as well as CaN inhibition in PBMCs, and that CaN activity at trough time points is a single surrogate predictor for the overall CaN activity throughout 12-hour and 24-hour dosing intervals in LDLT patients given cyclosporine. In addition, the present study suggests that a once daily morning administration of cyclosporine may be a novel and

convenient strategy for achieving adequate cyclosporine exposure and effective CaN inhibition early after LDLT.

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Clinical Outcomes of Living Donor Liver Transplantation for Hepatitis C Virus (HCV)-Positive Patients

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Background. Whether hepatitis C virus recurrence occurs earlier and with greater severity for living donor liver transplantation (LDLT) than for deceased donor liver transplantation (DDLT) has recently become a subject of debate.
Methods. We retrospectively evaluated clinical outcomes for a cohort of 91 HCV-positive patients who underwent LDLT at Kyoto University with a median follow-up period of 25 months.
Results. Overall 5-year patient survival for HCV patients was similar to that for non-HCV patients (n=209) who underwent right-lobe LDLT at our institute (69% vs. 71%). Survival rate of patients without HCC (n=34) tended to be better than that of patients with HCC (n=57) (82% vs. 60%, $P=0.069$). According to annual liver biopsy, rate of fibrosis progression to stage 2 or more (representing significant fibrosis) was 39% at 2 years after LDLT. Univariate analysis showed that female recipient and male donor represented significant risk factors for significant fibrosis. Progression to severe recurrence (defined as the presence of liver cirrhosis (F4) in a liver biopsy and/or the development of clinical decompensation) was observed in five patients.
Conclusions. Postoperative patient survival was similar for HCV-positive and -negative recipients in our adult LDLT series. Rates of progression to severe disease due to HCV recurrence seemed comparable between our LDLT recipients and DDLT recipients described in the literature. Although longer-term follow-up is required, our results suggest that LDLT can produce acceptable outcomes also for patients suffering from HCV-related cirrhosis.

Keywords: Hepatitis C virus, Living donor, Recurrence.

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Chronic hepatitis C virus (HCV) has become a global epidemic, with an estimated 200 million people currently infected worldwide. Nowadays, HCV-related cirrhosis is the most common indication for liver transplantation. However, recurrence of HCV infection is universal and often occurs immediately after transplantation (*1*). The prevalence of chronic hepatitis C in HCV-positive liver transplant recipients is 70–90% after 1 year, and rate of fibrosis progression is accelerated so that 20–40% of patients progress to allograft cirrhosis within 5 years (*2–6*). As a result, graft and patient survival is significantly reduced for HCV-positive recipients compared with HCV-negative recipients (*6–7*).

In Japan, too, HCV-related cirrhosis and hepatocellular carcinoma (HCC) represent the most prevalent liver diseases, and living donor liver transplantation (LDLT) has become a treatment option for patients with these diseases. However, a warning was recently issued by some Western transplant centers that HCV recurrence may occur earlier and with greater severity, and graft loss caused by recurrent HCV may be more frequent for LDLT than for deceased donor liver transplantation (DDLT) (*8–11*). Some suggestions have been offered for mechanisms that could increase graft damage in HCV-infected LDLT recipients (*12*). First, because the right hepatic lobe graft undergoes intense regeneration immediately after LDLT, specific cellular changes occurring during this vigorous proliferative response may facilitate entry of HCV into hepatocytes or promote HCV replication. Second, since most living donors are primary relatives of the recipient, increased genetic similarity and a higher degree of HLA matching between donor and recipient compared with DDLT may affect the severity of recurrent HCV infection. Conversely, more recent studies have reported comparable results between LDLT and DDLT (*13–15*). Such discrepancies may be explained in part by the small numbers of LDLT patients included in these studies, or learning curve effects on recent data associated with increased experience (*16*).

This issue has attracted worldwide attention because, given the shortage of deceased donor organs, increasing numbers of patients are choosing to undergo LDLT. The matter is of critical importance in Japan and countries where almost all liver transplantations use living donor grafts. The present study retrospectively evaluated clinical outcomes for a comparatively large cohort of 91 patients who underwent LDLT for HCV-related cirrhosis at our institute. We investigated the frequency and severity of posttransplant recurrence of chronic HCV hepatitis and examined risk factors in order to clarify the role of LDLT in the treatment of patients with HCV cirrhosis.

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PATIENTS AND METHODS

Patients

Between March 1999 and April 2005, LDLT was performed at Kyoto University on 105 patients with HCV cirrhosis. Of these, 91 patients (61 men, 30 women) who had undergone LDLT by June 2004 and had been followed up for >12 months were included in this study (Table 1). Median age of subjects was 55 years (range, 30–69 years). Median model for end-stage liver disease (MELD) score was 16 (range, 4–33). HCV cirrhosis was accompanied by HCC in 57 patients (63%), including 25 who exceeded Milan criteria. Median MELD scores in groups with and without HCC were 15 (range, 4–33) and 18 (range, 9–33), respectively (P=0.015, Mann-Whitney U test).

All patients were positive for anti-HCV antibody before the operation. Preoperative HCV RNA load, measured using the polymerase chain reaction (PCR) method with an Amplicor HCV assay (Roche Molecular Systems, Pleasanton, CA), was obtained for 74 patients, with a median value of 260 kIU/ml (range: <0.5–2400). Patients treated during the early period, in whom viral load was measured only using DNA probe methods, were considered to lack relevant data. HCV genotype, determined using a system based on PCR with genotype-specific primers (*17*), was: 1b (n=52); 2a (n=6); 2b (n=3); others (n=2); not determined (low viral load, n=3); or not examined (n=25).

LDLT using a right-lobe graft was performed on all except two patients who received left lobe grafts. Operative procedures for donor and recipient surgery have been described elsewhere (*18*, *19*). Donors were 52 men and 39 women, with a median age of 40 years (range, 19–64 years). Relationship to the recipient was: child (n=36); spouse (n=34); sibling (n=17); parent (n=1); or other (n=3). ABO blood-type matching was incompatible in 15 cases.

After discharge, patients were scheduled for monthly visits to the outpatient clinic for the first year. Median duration of follow-up was 25 months (range, 1–72 months).

TABLE 1. Preoperative profile and clinical characteristics

Characteristic	Data
n	91
Recipient sex (male/female)	61/30
Median recipient age, years (range)	55 (30–67)
Child-Pugh grade (A/B/C)	2/26/63
Median MELD score (range)	16 (4–33)
Pretransplant HCC (yes/no)	57/34
HCV genotype (1b/2a/2b/others)	52/6/3/2
Median HCV-RNA, kIU/mL (range)	260 (<0.5–2400)
Pretransplant interferon therapy (yes/no)	30/61
Median donor age, years (range)	40 (19–64)
Donor gender (male/female)	52/39
Relation to recipient (related/unrelated)	57/34
ABO blood-type mismatch (yes/no)	15/76
HLA-A,B mismatch (≤2/≥3)	70/21
HLA-DR 2 mismatch (yes/no)	26/65
GRWR ≥1.0% (yes/no)	54/37
Immunosuppression (FK/CyA)	83/6
Steroid-free induction (yes/no)	19/72
Methylprednisolone boluses for rejection (yes/no)	32/59

Immunosuppression

The standard immunosuppression protocol comprised tacrolimus and low-dose steroid (*20*). The target whole-blood trough level for tacrolimus was 10–15 ng/ml during the first 2 weeks, approximately 10 ng/ml thereafter, and 5–8 ng/ml from the second month. Cyclosporine microemulsion was administered instead of tacrolimus for induction immunosuppression in six patients. Steroid therapy was initiated at a dose of 10 mg/kg before graft reperfusion, then tapered from 1 mg/kg/day on day 1 to 0.3 mg/kg/day until the end of the first month, followed by 0.1 mg/kg/day until the end of the third month. After this time, steroid administration was terminated. As an exception, 13 patients received steroid-free tacrolimus monotherapy as an induction procedure in an attempt to reduce HCC recurrence. In addition, four patients were assigned to a tacrolimus plus mycophenolate mofetil (MMF) (without steroid) group in a prospective comparative study started in March 2004 to evaluate the effects of steroid-free immunosuppression on recurrence of HCV. Another two patients transplanted with grafts from an identical twin did not receive any immunosuppressive treatment.

Patients who received ABO blood-type incompatible transplants were treated with preoperative plasma exchange or double-filtration plasmapheresis in order to reduce anti-A or B antibody titers. During the first 3 weeks postoperatively, prostaglandin E1 and additional steroids were administered via the portal vein or hepatic artery (*21*). Cyclophosphamide was also given intravenously for the first 2 weeks, and then orally.

Acute rejection episodes were documented by means of liver histology (*22*) and treated with methylprednisolone boluses if moderate or severe. OKT-3 was used for only one patient. MMF or azathioprine was added for patients who experienced refractory rejections or required reduction of tacrolimus dose due to adverse effects.

Antiviral Therapy

Prophylactic antiviral therapy for HCV was not administered. As a rule, antiviral treatment was used for patients with recurrent chronic hepatitis C. The treatment protocol consisted of interferon α2b ($3–6\times10^6$ units 3 times/week) plus ribavirin (400-800 mg/day orally for the first 6 months), followed by interferon monotherapy for 6 months.

Histological Assessment

A total of 398 liver biopsies were evaluated when patients displayed liver enzyme levels elevated more than two to three times the normal upper limit, or at yearly intervals when informed consent was obtained. Annual follow-up biopsies were obtained from 60 patients at 1 year after LDLT, 34 patients at 2 years, 14 patients at 3 years, 6 patients at 4 years, and 1 patient at 5 years. Biopsy specimens were evaluated by a single pathologist (H.H.) with extensive experience in the pa-

thology of liver transplantation. Necroinflammatory activity (A0-A4) and fibrosis stage (F0-F4) were assessed using the METAVIR score (*23*, *24*). Fibrosis of stage 2 or higher was defined as significant fibrosis and was used as one of the endpoints in this study. A stage score of 2 was considered easily separable from stage 1 as a dividing point, as stage 1 involves fibrosis confined to the portal tract.

Prognostic Factors for Patient Survival and HCV Recurrence

A total of 18 variables potentially associated with patient survival and HCV recurrence were evaluated. Pretransplantation variables included: recipient age; recipient gender; Child-Pugh grade; MELD score; presence of HCC; HCV genotype (1b vs. non-1b); HCV viral load; and history of previous antiviral treatment with interferon. Donor-related variables comprised: age; gender; relation to the recipient (related vs. unrelated); ABO-blood type and HLA compatibilities; graft-to-recipient body weight ratio (GRWR: $<1.0\%$ vs. $\geq 1.0\%$). Posttransplant variables were: induction immunotherapy (tacrolimus vs. cyclosporine, with or without steroid); and administration of steroid boluses.

Statistical Analysis

Overall survival, time to reach fibrosis of stage 2 or more according to liver biopsy (with time to last biopsy for all patients who did not reach fibrosis of stage 2), and time to severe HCV recurrence were evaluated. Severe HCV recurrence was defined as the presence of liver cirrhosis (F4) in a liver biopsy and/or the development of clinical decompensation secondary to liver diseases with portal hypertension (*11*). Cumulative probability curves of survival or HCV recurrence were calculated using the Kaplan-Meier method, and differences between these curves were compared using the log-rank test. The cutoff chosen for quantitative variables was the median, unless stated otherwise. Any variable identified as significant ($P<0.05$) in univariate analysis by log-rank testing was considered a candidate for multivariate analysis using Cox's proportional hazards regression model. Values of $P<0.05$ were considered statistically significant.

RESULTS

Patient Survival

As of the end of May 2005, 65 patients were still alive. One patient had received re-LDLT for graft cirrhosis due to HCV recurrence 31 months after first LDLT and has survived 14 months since then. Causes of death for the 26 patients were: sepsis (n=11); peritonitis (n=4); pneumonia (n=3); recurrent HCC (n=4); chronic rejection (n=2); veno-occlusive disease (n=1); and recurrent HCV (fibrosing cholestatic hepatitis (FCH), n=1). Overall patient survival rate at 5 years was 69%, similar to that of 209 non-HCV patients (71%) who underwent right-lobe LDLT at our institute between February 1998 and June 2004 (PBC/PSC, n=56; HBV cirrhosis, n=53; fulminant hepatitis, n=38; cholestatic disease, n=19; and others, n=43; Fig. 1). Among HCV-positive patients, 5-year survival rate tended to be better in patients without HCC (n=34) than in patients with HCC (n=57), although no significant difference was identified (82% vs. 60%, $P=0.069$; Fig. 2). None of the variables listed above as prognostic factors for patient survival and HCV recurrence displayed any significantly associations with patient survival.

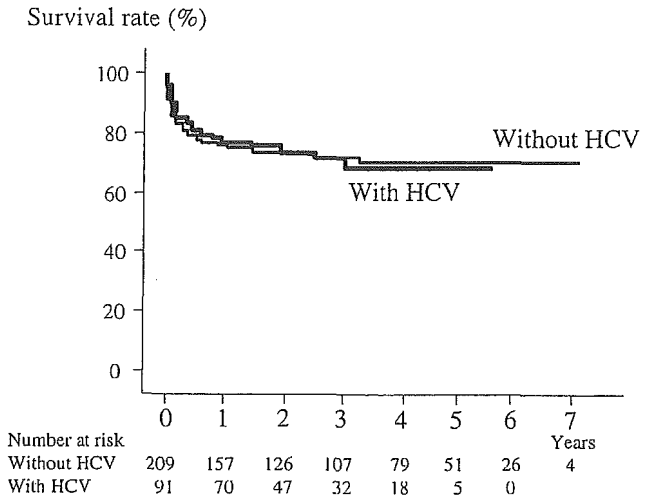

FIGURE 1. Patient survival after living donor liver transplantation (HCV vs. non-HCV). Overall patient survival rate for HCV-positive patients was 69% at 5 years, similar to that for non-HCV patients (71%, n=209) who underwent right-lobe LDLT at our institute between February 1998 and June 2004.

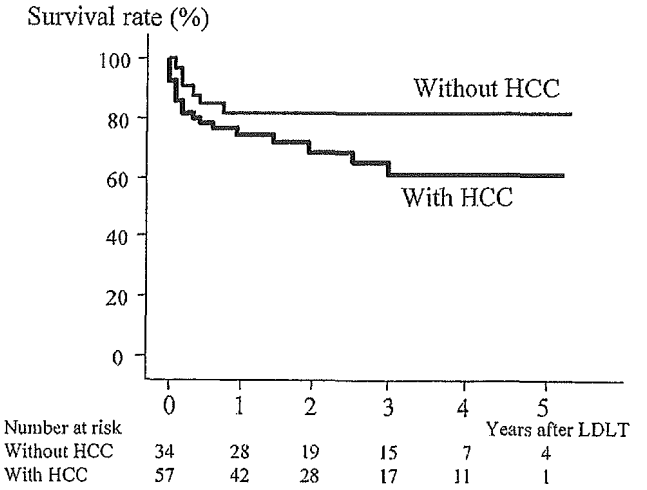

FIGURE 2. Patient survival for HCV patients (with vs. without HCC). Among HCV-positive patients, survival rate tended to be better for patients without HCC (n=34) than for patients with HCC (n=57), although no significant difference was found (82% vs. 60%, $P=0.069$).

Evaluation of Liver Histology

During the first year after LDLT, necroinflammatory changes suggesting recurrent hepatitis C were observed in 50 patients: A1 in 32 patients; A2 in 17 patients; and A3 in one patient. Afterwards, the percentage of patients who received biopsy among those alive at yearly intervals was as follows: 1 year, 86% (60/70); 2 years, 72% (34/47); 3 years, 44% (14/32); 4 years, 33% (6/18); and 5 years, 20% (1/5). Ten patients who were alive for >1 year (range, 22–72 months) without any

evidence of progressive liver disease never underwent any biopsy at 1 year or later. Significant fibrosis (stage 2 or more) was identified in 23 patients, including 3 patients who developed to fibrosis of stage 4 within 1 year. Excluding 19 patients who died within 1 year without identified fibrosis and the 10 patients alive without biopsy for >1 year, cumulative probability of significant fibrosis was 19% at 1 year after LDLT, 39% at 2 years, and 58% at 3 years. Follow-up was censored at the time of last biopsy for all patients who did not reach fibrosis of stage 2.

The results from univariate analysis of risk factors for significant fibrosis are summarized in Table 2. Female recipient and male donor were significantly associated with development of significant fibrosis. Analysis of quantitative variables, donor age and GRWR, demonstrated that rate of significant fibrosis was not significantly different even when cutoff levels were changed. Multivariate analysis with Cox's hazards model showed that neither female recipient nor male donor represented independent risk factors for significant fibrosis (data not shown).

Severe Recurrence of HCV

FCH was diagnosed in two patients, one of whom died of liver failure 7 months after LDLT. The other patient suffered from FCH 2 months after LDLT, but recovered from cholestasis and was still alive after 28 months. Final liver biopsies of both patients showed fibrosis of stage 4. Another three patients also developed fibrosis of stage 4 during follow-up. One patient whose liver biopsy led to a diagnosis of recurring chronic hepatitis with F3 fibrosis 10 month after LDLT also suffered from stenosis of duct-to-duct biliary anastomosis. This patient underwent hepaticojejunostomy, but died of fungal pneumonia 1 month later. Liver histology at autopsy revealed F4 fibrosis. Another patient received re-LDLT for recurrent decompensated cirrhosis, as described above. The other patient who developed to biopsy-proven stage 4 fibrosis at 50 months was alive without decompensation as of 63 months after LDLT. In total, severe recurrence (progression of biopsy-proven cirrhosis and/or occurrence of clinical decompensastion) was diagnosed in five patients, and cumulative probability of severe recurrence was 8% at 2 years. Of the five patients presenting with severe recurrence, three were female and all had received the liver graft from a male donor.

TABLE 2. Risk factors associated with fibrosis of stage 2 or higher

Factors	n	Recurrence rate (number of patients at risk) 1 year	2 years	3 years	*P* value
Total[a]	62	19% (50)	39% (14)	58% (5)	
Recipient sex					0.006
Male	40	10% (36)	27% (10)	48% (4)	
Female	22	36% (14)	60% (4)	70% (1)	
Donor sex					0.047
Male	36	25% (28)	49% (6)	59% (1)	
Female	26	12% (22)	26% (8)	47% (4)	

[a] The 19 patients who died within 1 year without identified fibrosis and the 10 patients alive without biopsy for >1 year were excluded.

DISCUSSION

In the present study, only one patient had died of recurrent HCV as of the time of writing, and the majority of posttransplant deaths were attributable to postoperative complications occurring within a few months after LDLT. Infectious complications such as sepsis, pneumonia and peritonitis represented the most common causes of early mortality, as was the case in HCV-negative recipients. One-year mortality rates were 23% and 25%, respectively. Currently, overall 5-year patient survival rate for HCV-positive patients appears similar to that for non-HCV patients in our adult LDLT series (69% vs. 71%; Fig. 1). Of the 91 patients, HCC was present in 57 (63%), including 25 patients who exceeded the Milan criteria. Four patients died of recurrent HCC after LDLT, and the survival rate tended to be lower for patients with HCC than for patients without HCC (82% vs. 74% at 1 year, and 82% vs. 60% at 5 years; Fig. 2). Only one patient in this cohort had to undergo re-transplantation, and 5-year graft survival rates were 68% for all patients and 82% for patients without HCC. These results are comparable to the reported DDLT outcomes in the UNOS database: patient and graft survival rates of HCV-positive patients (n=3955) at 2 years were 81% and 75%, respectively (*13*); and rates for HCV-positive but HCC-negative patients (n=5640) at 5 years were 74.6% and 69.9%, respectively (*7*).

Progression of fibrosis due to recurrent chronic hepatitis is key to determining graft prognosis after liver transplantation for HCV-positive recipients. In the present study, progression of fibrosis in the liver biopsy was assessed and fibrosis to stage 2 or more was defined as significant fibrosis. The probability of progression to significant fibrosis was 39% at 2 years after LDLT. Several risk factors associated with posttransplant recurrence of hepatitis C have been identified (*5, 25–27*). These include pretransplant viral load, genotype 1b, donor age and graft steatosis, recipient age, race, gender, coexistence of HCC, and rejection treatment using bolus steroid or antilymphocyte preparations. Among the 18 potential variables examined in our study, univariate analysis identified female recipient and male donor as closely related to significant fibrosis. However, multivariate analysis showed that neither variable represented a significant independent risk factors. Actually, some correlation among these two variables was noted. Of the 30 female recipients, 24 had received a liver graft from a male donor (son or husband). An association between female gender of the recipient and severity of recurrent HCV has been demonstrated in previous studies (*6, 7*). However, no previous reports have implicated gender of the donor as an involved factor. Although difficulty exists in determining which is the predominant factor, the combination of male donor and female recipient may exert a negative impact on HCV recurrence.

Rapid proliferation of hepatocytes during postoperative graft regeneration may contribute to a higher rates of both HCV replication and severe recurrence in LDLT (*11*). This seems to imply a higher risk of recurrence in cases involving smaller grafts, which are supposed to undergo regeneration at a higher rate. Our study, however, showed that progression of significant fibrosis was similar for patients who received grafts with GRWRs of <1.0% or ≥1.0%. This result is supported by a recent report (*28*) showing that liver

regeneration following partial liver transplant does not increase the risk of HCV recurrence. Likewise, neither the relationship between donor and recipient nor degree of HLA matching seemed to influence recurrence. The results of our study thus do not support the hypothesis that these factors may exert negative effects on HCV recurrence in LDLT patients.

Due to the small number of patients treated using DDLT in Japan, HCV recurrence rates could not be compared between DDLT and LDLT. In previous studies on HCV recurrence after DDLT (*1, 13, 25, 29, 30*), histologically diagnosed recurrence of chronic HCV occurred in 65–90% of HCV-positive DDLT recipients during the first 2 years. However, a lack of uniform definitions for recurrent HCV, even when histological liver biopsy findings are used as criteria, has been indicated as one reason for the difficulties in comparing studies on HCV recurrence (*31*). Recently, a report from Spain demonstrated that severe recurrence of hepatitis C, defined as the development of cirrhosis or clinically decompensated liver disease, is more frequent in LDLT recipients (*11*). According to this report, the 2-year probability of developing severe recurrence was 45% after LDLT, compared to 22% after DDLT ($P=0.019$). When the same definitions were applied, rate of severe recurrence was only 8% at 2 years in our study. Arguably as many as 19 patients (21%) died within 1 year before developing HCV recurrence in our series. However, considering that the probability of either death or severe recurrence was 29% at 2 years, the results for our LDLT series were not likely to be greatly inferior to other reported cases.

In conclusion, postoperative patient survival was similar for HCV-positive and -negative recipients in our adult LDLT series. Rate of recurrence for chronic HCV and prevalence of progression to severe disease for our LDLT recipients appeared comparable to those for DDLT reported in the literature. Although these results need to be confirmed with a longer follow-up period, the present findings suggest that LDLT can produce acceptable outcomes for patients suffering from end-stage liver disease due to chronic HCV.

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肝細胞癌の再発は必ず起こるのか

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1 はじめに

これまでの肝細胞癌に対する治療は併存する慢性肝障害のために治療法の選択が制限され，また肝内転移や肝炎ウィルスに関連した多中心性発癌による再発が高率である．一方，肝移植は癌病変の除去と同時にその背景にある慢性肝疾患を根本的に治療できるという利点がある．最近本邦でも成人に対する生体肝移植が広く行われるようになり，肝細胞癌に対しても生体肝移植が導入されている．しかし，移植後の癌再発は術後成績に大きな影響を与え，特に進行癌ほど再発率が高いことが示されている．本稿では，欧米での脳死肝移植における術後再発に関する知見を概略し，また筆者らの施設でのこれまでの肝細胞癌に対する生体肝移植の成績を紹介する．

2 肝細胞癌に対する脳死肝移植

欧米での脳死肝移植では，初期の頃は切除不能進行肝細胞癌に対して積極的に肝移植が行われたが，術後再発が多く5年生存率は15～30％とその成績は不良であった[1-3]．そして，肝移植実施数の増加に伴い移植臓器不足や医療経済などの問題が深刻化し，適応の見直しがはかられた．その中で1996年にMazzaferroらが[4]，術前画像診断で脈管浸潤およびリンパ節転移がなく，腫瘍が単発ならば直径5 cm以下，多発ならば3個以下で最大径が3 cm以下の場合，75％の生存率が得られると報告した．これらの条件，いわゆる「ミラノ基準」，を満たす症例は肝癌以外の移植症例と同等の成績が得られることが示され，その後世界的にもこの基準が肝細胞癌に対する移植適応として一般的に受け入れられるようになり，本邦の脳死肝移植の適応基準にも採用されている．このような背景には，数に限りのある移植臓器を有効に利用するためには，再発の危険性が少ない症例を移植適応とするべきであるという考え方が存在している．

一方で，このミラノ基準を超えた症例でも同等の成績が期待できる可能性があり，より多くの肝癌患者を救命することを目的として

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