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Note

Interaction between Tacrolimus and Lansoprazole, but not Rabeprazole in Living-Donor Liver Transplant Patients with Defects of *CYP2C19* and *CYP3A5*

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Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: We report different effects of administration of proton pump inhibitors on tacrolimus blood concentration in two living-donor liver transplant patients. In case 1, a 51-year-old man with liver cirrhosis due to hepatitis C virus underwent living-donor liver transplantation, and tacrolimus was orally administered. Omeprazole (40 mg/day) was introduced intravenously between postoperative days 5 and 6, and oral lansoprazole (30 mg/day) was introduced from day 6, leading to an increase in the concentration/dose ratio of tacrolimus from day 10. In case 2, a 41-year-old living-donor liver transplant woman received tacrolimus, and co-administered with omeprazole (40 mg/day) intravenously during 7 days immediately after surgery. During this period, trough concentration of tacrolimus was high, but the concentration/dose ratio of tacrolimus was gradually decreasing with time. Switched to rabeprazole (10 mg/day) orally on the postoperative 8th day, the concentration/dose ratio of tacrolimus remained low, indicating little drug-drug interaction between tacrolimus and rabeprazole. In both cases, the genotypes of *CYP2C19* and *CYP3A5* were defective both in the graft liver and in the native intestine. A drug-drug interaction between rabeprazole and tacrolimus was not observed in this case study presented, suggesting that this combination could be safely used in tacrolimus therapy after liver transplantation.

Keywords: CYP2C19; CYP3A5; living-donor liver transplantation; tacrolimus; lansoprazole; rabeprazole

Introduction

An immunosuppressant tacrolimus is widely used in organ transplantation. The systemic clearance of tacrolimus is mainly explained by cytochrome P450 (CYP) 3A4/5-mediated metabolism in the liver and small intestine.[1)] Proton pump inhibitors (PPIs), lansoprazole and rabeprazole, are empirically co-administered with tacrolimus in patients suffering from surgical stress-related gastric bleeding or gastrointestinal ulceration after organ transplantation. PPIs are primarily metabolized by CYP2C19 and CYP3As.[2,3)] Interaction between tacrolimus and PPIs is therefore assumed to occur especially in patients with *CYP2C19* gene variants. However, no studies have investigated the effect of PPIs on tacrolimus pharmacokinetics in living-donor liver transplant (LDLT) patients.

We herein report the different effects of PPIs on tacrolimus pharmacokinetics in LDLT patients with *CYP2C19* and *CYP3A5* defect genotypes in both the graft liver and the native intestine.

Methods

Ethics: These studies were conducted in accordance with the Declaration of Helsinki and its amendments, and

Received; September 10, 2007, Accepted; November 20, 2007
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Abbreviations: CYP, cytochrome P450; PPIs, Proton pump inhibitors; LDLT, living-donor liver transplantation.
Sponsorship: This work was supported in part by the 21st Century Center of Excellence Program "Knowledge Information Infrastructure for Genome Science"; by a grant-in-aid from the Japan Health Sciences Foundation ("Research on Health Sciences Focusing on Drug Innovation"); by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan; and by the 21st Century COE program "Knowledge Information Infrastructure for Genome Science". K. Hosohata was supported as a Research Assistant by the 21st Century COE program "Knowledge Information Infrastructure for Genome Science"

were approved by the Kyoto University Graduate School and Faculty of Medicine, Ethics Committee. Written informed consent was obtained from the recipients.

Dosage regimen of tacrolimus, analysis of blood samples: The basic immunosuppression regimen consisted of tacrolimus with low-dose steroids, methylprednisolone (Solu-Medrol®; Pfizer, New York, USA) and prednisolone (Predonine®; Shionogi, Osaka, Japan). The blood concentrations of tacrolimus (Prograf®; Fujisawa Pharmaceutical Co. Ltd, Osaka, Japan), under co-administration of omeprazole (Omepral®, AstraZeneca Co. Ltd, Osaka, Japan), lansoprazole (Takepron®, Takeda Pharmaceutical Co. Ltd, Osaka, Japan) and rabeprazole (Pariet®, Eisai Co. Ltd, Tokyo, Japan), were monitored using a semiautomated microparticle enzyme immnoassay (IMx®; Abbott Co, Ltd, Tokyo, Japan).

Isolation of genomic DNA and genotyping: *CYP2C19* and *CYP3A5* genotypings were performed using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method,[4,5] with genomic DNA extracted from a liver biopsy specimen of the graft and peripheral blood from recipients by using a MagNAPure LC DNA Isolation kit I (Roche, Mannheim, Germany). The PCR products were digested with a restriction enzyme according to the condition of manufacturers' instructions and separated on 3.5% agarose gel.

Results

Case report 1: Case 1 was a 51-year-old man weighing 84 kg who underwent a LDLT for hepatocellular carcinoma and liver cirrhosis due to hepatitis C virus. The graft-to-recipient body weight ratio (GRWR) was 0.99% and the blood type combination was identical (A+ to A+). Primary immunosuppression consisted of tacrolimus, prednisolone, and mycophenolate mofetil. Within 24 hours after surgery, administration of tacrolimus was started with an oral dose of 0.075 mg/kg twice daily (at 9 AM and 9 PM). The dosage of tacrolimus was adjusted to reach whole-blood trough

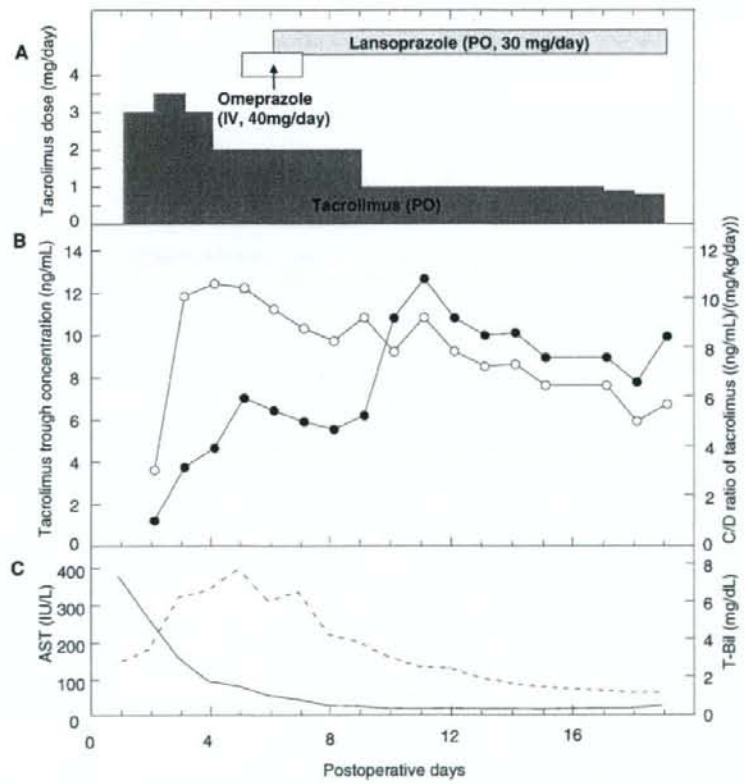

Fig. 1. Monitoring tacrolimus oral doses (A), trough concentrations (B, open circles) and C/D (B, concentration/dose, solid circles) ratio, and serum AST (aspartate aminotransferase, solid line) and T-Bil (total bilirubin, dashed line) levels (C) before and after the co-administration of lansoprazole with tacrolimus in patient 1.
C/D, concentration/dose; AST, aspartate aminotransferase; T-Bil, total bilirubin; IV, intravenous administration.

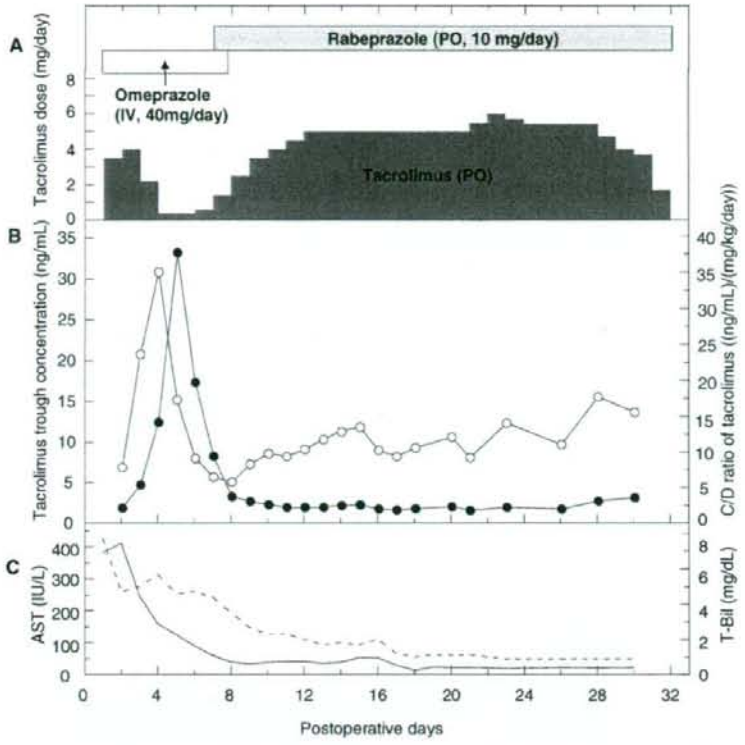

Fig. 2. Monitoring tacrolimus oral doses (A), trough concentrations (B, open circles) and C/D (B, concentration/dose, solid circles) ratio, and serum AST (aspartate aminotransferase, solid line) and T-Bil (total bilirubin, dashed line) levels (C) before and after the co-administration of rabeprazole with tacrolimus in patient 2.
C/D, concentration/dose; AST, aspartate aminotransferase; T-Bil, total bilirubin; IV, intravenous administration.

concentrations ranging between 5 and 15 ng/mL during the first month. As prophylaxis for ulcer, omeprazole at 40 mg/day was administered intravenously for the period of days 5–6, swiched to lansoprazole at 30 mg/day between days 6 and 19 (**Fig. 1A**), leading to an increase in the concentration/dose (C/D) ratio of tacrolimus. Graft liver function, as assessed based on the aspartate aminotransferase level and total bilirubin level, almost returned to normal during the first postoperative month (**Fig. 1C**). No other clinical events affecting the level of tacrolimus was reported. He had heterozygous variants at exons 4 and 5 of *CYP 2C19* (*CYP2C19*2/*3*), which showed him to be a poor metabolizer. The donor was his 55-year-old brother with *CYP2C19*2/*3*. Furthermore, the genotype of *CYP3A5* in both the graft liver (donor) and native intestine (recipient) was *CYP3A5*3/*3* (CYP3A5 non-expressors).

Case report 2: Case 2 was a 41-year-old woman with liver cirrhosis of unknown etiology, who underwent a LDLT. The GRWR was 1.46% and the blood type combination was incompatible (B+ to A+). The recipient received triple-regimen-therapy including tacrolimus, corticosteroid, and mycophenolate mofetil. A standard corticosteroid tapering regimen was used, consisting of an intravenous bolus of methylprednisolone at 50 mg/day on day 1, 25 mg/day between days 4 and 6, and 15 mg/day on day 7, followed by oral prednisolone at 15 mg/day between days 8 and 28, and 5 mg/day on days 29 and 30. As prophylaxis for ulcer, omeprazole at 40 mg/day was intravenously administered during 7 days immediately after the surgery. During this period, the trough concentration of tacrolimus was high, but the C/D ratio of tacrolimus was gradually decreasing with time and/or grafted liver regeneration from postoperative day 5 (**Figs. 2A, 2B**). On the postoperative 8th day, there was a switch from omeprazole at 40 mg/day intravenously to rabeprazole at 10 mg/day orally, but the C/D ratio of tacrolimus remained low. There was little drug interaction between tacrolimus and rabeprazole (**Figs. 2A, 2B**). No other symptoms of liver impairment including jaundice or acute cellular rejection were observed. She had homogenous variants at exon 5 of *CYP2C19* (*CYP2C19*2/*2*), which showed her to be a poor metabolizer. The donor was her 63-year-old mother with *CYP2C19*2/*3*. The geno-

type of *CYP3A5* in both the graft liver (donor) and native intestine (recipient) is *CYP3A5*3/*3* (CYP3A5 non-expressors).

Discussion

This is the first report that the effect of PPI on tacrolimus pharmacokinetics between two LDLT cases, whose genotypes of *CYP2C19* and *CYP3A5* were defective in both the graft liver and the native intestine. In patient 1, omeprazole was administered intravenously between days 5 and 7 after surgery and switched to oral lansoprazole from day 6, leading to an increase in the C/D ratio of tacrolimus. No alterration of liver function was noted, and no other medication or clinical events such as acute cellular rejection was reported. This observation suggests the drug-drug interaction between lansoprazole and tacrolimus.

In contrast, the effect of PPIs on tacrolimus pharmacokinetics varied in patient 2. The C/D ratio of tacrolimus was high during 7 days immediately after surgery, but after that, it was gradually decreased. The C/D ratio of tacrolimus remained low under co-administration of rabeprazole, indicating little effect of rabeprazole on the metabolism of tacrolimus.

The administration of oral lansoprazole or rabeprazole after intravenous omeprazole showed different effects on tacrolimus pharmacokinetics. One possible explanation for this difference is that the relative contribution of CYP2C19-mediated metabolism varies between the two PPIs.[2,3] Rabeprazole is disposed mainly via a non-enzymatic pathway to thioether-rabeprazole, with only a minor pathway via CYP2C19 and CYP3A4.[3] Another possibility is that this difference can be explained by an inhibition of P-glycoprotein-mediated intestinal efflux-transport of tacrolimus by lansoplazole,[6] but not rabeprazole.[7] P-glycoprotein is related to the inter-individual variation in the pharmacokinetics of tacrolimus as an absorptive barrier.[8-12] Therefore, the intestinal expression level of P-glycoprotein can limit bioavailability of tacrolimus in patient 1.

To date, no interactions between lansoprazole and tacrolimus in LDLT patients have been demonstrated. Several reports stated that lansoprazole interfered with the metabolism of tacrolimus in renal transplant patients with *CYP2C19* gene variants.[13,14] These reports showed that the CYP3A4-mediated metabolism of tacrolimus may be competed with lansoprazole, but there were no examinations of the polymorphism in the *CYP3A5* gene, which could not exclude the potential contribution of CYP3A5 to the interaction between tacrolimus and lansoprazole. In our report, the defective genotypes of *CYP3A5* as well as *CYP2C19* in both the graft liver and the native intestine could reveal the CYP3A4-mediated drug interaction between tacrolimus and lansprazole more clearly. However, the contribution of CYP3A5 to the interaction between tacrolimus and lansoprazole or rabeprazole remains unclear. Because the genotypes of *CYP3A5* significantly affected on the tacrolimus pharmacokinetics in LDLT patients,[15] the interaction between tacrolimus and PPIs should be examined in future. In addition, further research is needed to confirm what extent rabeprazole is metabolized by CYP3A5 in vitro study.

In conclusion, we first found drug-drug interaction between tacrolimus and lansoprazole, but not rabeprazole, in living-donor liver transplant patients with genetic defects of *CYP2C19* and *CYP3A5*, suggesting that rabeprazole could be safely used in tacrolimus therapy after the liver transplantation.

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0022-3565/08/3252-610–616$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 325:610–616, 2008

Vol. 325, No. 2
135665/3326443
Printed in U.S.A.

Relation between mRNA Expression Level of Multidrug Resistance 1/ABCB1 in Blood Cells and Required Level of Tacrolimus in Pediatric Living-Donor Liver Transplantation

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Received December 19, 2007; accepted February 1, 2008

ABSTRACT

It has been difficult to set an individualized therapeutic window of tacrolimus after organ transplantation, because of wide interindividual variation of responsiveness to immunosuppressive therapy. In this study, we examined the significance of multidrug resistance 1 (MDR1) in the peripheral blood cells by comparing the trough concentration of tacrolimus with the occurrence of acute cellular rejection (ACR) in retrospectively collected pediatric living-donor liver transplant patients, who were enrolled after obtaining written informed consent. No significant difference in the intraindividual variation in MDR1 mRNA expression in the peripheral blood cells was observed between postoperative days 3 and 7. The average trough concentration of tacrolimus during the 15-day postoperative period was significantly higher in the event-free patients than in those who experienced ACR (21 of 44 cases), and they had higher levels of blood MDR1 mRNA. In addition, the average trough concentration of tacrolimus significantly correlated with the logarithmically transformed MDR1 mRNA data from the blood cells in patients of both the event-free ($r = 0.5406$; $P = 0.0077$) and ACR ($r = 0.4772$; $P = 0.0284$). The cellular accumulation of [^{14}C]tacrolimus in the peripheral blood mononuclear cells was 2-fold higher in *mdr1a/1b*-knockout mice than in wild-type mice ($P = 0.0182$). These results suggest that MDR1 in blood cells decreases the leukocytic concentration of tacrolimus, and it could be a useful marker to establish an individualized target concentration of tacrolimus to prevent ACR in pediatric patients after liver transplantation.

Tacrolimus, a calcineurin inhibitor, is widely used as an immunosuppressant in patients undergoing organ transplantation. It acts on T lymphocytes and inhibits the nuclear factor of activated T cells-derived production of interleukin 2 and the subsequent proliferation of lymphocytes, thereby contributing to a marked improvement in graft survival (Denton et al., 1999; Scott et al., 2003). The therapeutic range of tacrolimus for liver transplantation is considered to be approximately 5–20 ng/ml, but the blood concentration of tacrolimus during the development of acute cellular rejection (ACR) differs among patients (Yasuhara et al., 1995). Because no sensitive marker of drug effectiveness has yet been identified, the establishment of a dosage regimen based on individual susceptibility is required.

P-glycoprotein is the product of multidrug resistance gene *MDR1/ABCB1*. It exists in several tissues such as the intestine, liver, and kidney and prevents the intracellular accumulation of numerous drugs (Cordon-Cardo et al., 1990; Hoffmann and Kroemer, 2004). P-glycoprotein in the intestine and kidney is considered to influence the pharmacokinetics of various types of drugs during absorption and tubular excretion, and some drug interactions via the transporter have also been reported (Wakasugi et al., 1998; Greiner et al., 1999). It is also expressed in various types of blood cells; depending on its expression level, it is reported to prevent the uptake of doxorubicin (Klimecki et al., 1994). Furthermore, it has also been reported as a prognostic factor to predict relapse of childhood acute lymphoblastic leukemia (Dhooge et al., 1999). Based on these findings, it is hypothesized that P-glycoprotein in the peripheral blood cells also decreases the leukocytic concentration of tacrolimus, and its expression

This work was supported in part by a grant-in-aid from Japan Health Sciences Foundation for "Research on Health Sciences Focusing on Drug Innovation"; a grant-in-aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan; and the 21st Century COE Program "Knowledge Information Infrastructure for Genome Science."
Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.
doi:10.1124/jpet.107.135665.

ABBREVIATIONS: ACR, acute cellular rejection; MDR, multidrug resistance; LDLT, living-donor liver transplantation; PBMC, peripheral blood mononuclear cell.

level is related to the frequency of ACR in liver transplant patients.

In the present study, we examined the relationship among the MDR1 mRNA expression level in the peripheral blood cells, tacrolimus trough concentration, and occurrence of ACR in pediatric patients undergoing living-donor liver transplantation (LDLT). Furthermore, we compared the intracellular concentration of tacrolimus in the peripheral blood mononuclear cells (PBMCs) between *mdr1a/1b* null mice and wild-type mice.

Materials and Methods

Patients, Dosage Regimen of Tacrolimus, Analysis of Blood Samples, and Criteria for ACR. Between October 2001 and September 2003, 44 pediatric patients who underwent LDLT in Kyoto University Hospital were enrolled in this study after written informed consent was obtained from them and/or their parents. The patients were aged between 0.25 and 14 years. The primary diseases were biliary atresia (33 patients, including one patient with a liver tumor and one patient with Alagille syndrome), Alagille syndrome (two patients), propionic acidemia (two patients), Wilson disease (one patient), primary sclerosing cholangitis (one patient), Byler disease (one patient), and re-LDLT (four patients). The blood type matches between the donors and recipients were identical in 34 cases and compatible in 10 cases. The demographics of the control event-free group and the ACR group are shown in Table 1. This study was conducted in accordance with the Declaration of Helsinki and its amendments, and it was approved by the Ethics Committee of Kyoto University.

After transplantation, immunosuppression by oral administration of tacrolimus (0.04 mg/kg) every 12 h was initiated at 12 h after reperfusion (Asonuma et al., 1998). The target trough blood concentration of tacrolimus was 10 to 12 ng/ml during the first 2 weeks after transplantation. The daily oral dose was modulated based on the whole blood tacrolimus concentration that was measured approximately 12 h after the evening administration by using a semiautomated microparticle enzyme immunoassay (IMx; Abbott, Tokyo, Japan) (Yasuhara et al., 1995).

ACR was defined by the biochemical abnormalities that were mainly evaluated by the re-elevation of transaminases and by the histological evaluation of liver biopsy specimens. The patients who were diagnosed with ACR were treated with a high-dose i.v. administration of methylprednisolone or corticosterone.

Evaluation of MDR1 mRNA Expression Level in Blood. The blood samples for the examination of mRNA expression were collected in a PAXgene Blood RNA tube (QIAGEN Gmbh, Hilden, Germany) on postoperative days 3 and 7. The total RNA was extracted using the PAXgene Blood RNA kit (QIAGEN), and they were reverse transcribed by SuperScript II transcriptase (Invitrogen, Carlsbad, CA) after the digestion of contaminated genomic DNA by RQ1 DNase (Promega, Tokyo, Japan), as described previously (Masuda et al., 2000). The mRNA expression level of MDR1 was quantitated by real-time PCR using the Prism 7700 sequence detection system (Applied Biosystems, Foster City, CA). The primer and TaqMan probe sets used have been described previously (Uwai et al., 2004).

Distribution of [^{14}C]Tacrolimus in PBMCs in *mdr1a/1b* Null Mice. FVB control wild-type mice and *mdr1a/1b* double-knockout mice, weighing 30 to 40 g (Taconic Farms, Albany, NY), were used. Before the experiments, the mice were housed in a temperature- and humidity-controlled room, and they were allowed free access to water and standard chow. The animal experiments were performed in accordance with the Guidelines for Animal Experiments of Kyoto University. The experimental protocol was approved by the Animal Research Committee (Graduate School of Medicine, Kyoto University, Kyoto, Japan).

After diethyl ether anesthesia, whole blood sample was collected from the aorta, and biochemical parameters were confirmed using the I-STAT analyzer (Abbott). The mean hematocrit values of the FVB control mice ($n = 7$) and *mdr1a/1b* null mice ($n = 8$) were 32.9% and 30.9%, respectively ($P = 0.3315$ by Student's t test). The blood levels of Na^+, K^+, Cl^-, blood urea nitrogen, and glucose (data not shown) did not exhibit abnormal values. The [^{14}C]tacrolimus (683 kBq/mg; kindly provided by Astellas Pharma Co., Tokyo, Japan) was spiked in the whole blood (6.4 kBq/ml; 10.4 μg/ml), and then it was incubated at 37°C for 30 min with gentle shaking. At the end of the incubation, the whole blood was diluted with the same volume of phosphate-buffered saline, pH 7.4, to isolate PBMCs by using Ficoll-Paque Plus (GE Healthcare Biosciences, Tokyo, Japan) (Fukudo et al., 2005a,b). The contaminating red blood cells were removed with red blood cell lysis buffer (Roche Diagnostics KK, Tokyo, Japan). The PBMC samples were dissolved using 0.5 ml of NCS tissue solubilizer (GE Healthcare Biosciences), and liquid scintillation counting was performed with 3 ml of the ACSII scintillation cocktail (GE Healthcare Biosciences).

Statistics. Statistical analysis was performed using Prism version 4.0 software (GraphPad Software Inc., San Diego, CA). Logarithmic transformation of the mRNA levels of MDR1 was performed to improve normality before performing statistical analyses such as distribution examination and correlation analysis. Statistical differences between the two groups were analyzed by Student's t test and the Mann-Whitney U test after performing analysis of normality and the F test.

Results

MDR1 mRNA Expression Level in the Peripheral Blood Cells. Figure 1A shows a histogram of logarithmically transformed data representing the expression levels of MDR1 mRNA in the peripheral blood cells at postoperative day 3. The median values of MDR1 mRNA in the all patients at postoperative days 3 and 7 were 0.05 and 0.09 amol/μg total RNA, respectively. The MDR1 mRNA level in the peripheral blood cells was compared between postoperative days 7 and 3 in each of the 32 patients (Fig. 1B). Because the high-dose steroid pulse therapy was administered between

TABLE 1
Demographics of patients

	Control (Event-Free)		Acute Cellular Rejection	
No. of patients		23		21
Age (yr)		0.25–12		0.5–14.1
Primary disease	Biliary atresia	16	Biliary atresia	15
	with liver tumor	1	Alagille syndrome	2
	with Alagille syndrome	1	Re-LDLT	1
	Re-LDLT	3	Primary sclerosing cholangitis	1
	Propionic acidemia	2	Byler disease	1
			Wilson disease	1
Blood type	Identical	18	Identical	16
	Compatible	5	Compatible	5

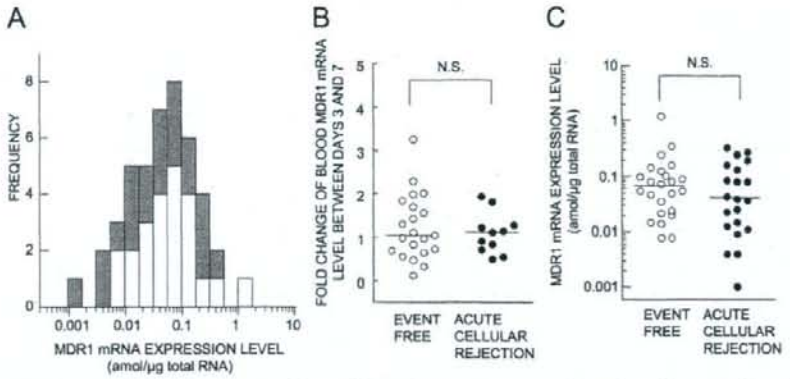

Fig. 1. Expression levels of MDR1 mRNA in the peripheral blood cells in pediatric patients after living-donor liver transplantation. A, histogram of the MDR1 mRNA expression level in the event-free patients (empty columns) and in those who experienced acute cellular rejection (filled columns) (n = 44). B, comparison of the MDR1 mRNA levels between postoperative days 3 and 7 (n = 32). The data are expressed as the -fold change at postoperative day 7 compared with postoperative day 3. Seven patients were excluded because of high-dose steroid therapy administered between postoperative days 3 and 7. The blood samples at postoperative day 7 could not be obtained from five patients due to patient refusal. C, there was no statistical difference with regard to the MDR1 mRNA level in the peripheral blood cells between the event-free patients and those who experienced acute cellular rejection. Statistical analysis was performed using the Mann-Whitney U test (B and C). However, the P values were greater than 0.05.

postoperative days 3 and 7 to seven patients, their MDR1 mRNA expression data were excluded. The blood samples of five patients could not be obtained due to patient refusal. The distribution of the MDR1 mRNA level exhibited a similar unimodal pattern in both control cases (event-free, empty columns) as well as in the ACR cases (filled columns) (Fig. 1, A and C); furthermore, there was no statistically significant difference in the MDR1 mRNA levels between postoperative days 3 and 7 (data not shown), and in the -fold changes of the MDR1 mRNA levels during postoperative days 3 and 7 between the control and ACR cases (Fig. 1B). Therefore, the molecular data at postoperative day 3 were used for further examinations.

Blood Concentration Profiles of Tacrolimus, MDR1 mRNA Level in Blood Cells, and ACR. The blood concentration profiles of tacrolimus during the 15-day postoperative period are shown in Fig. 2. The patients were categorized into the following four groups based on the mRNA expression level of MDR1 at postoperative day 3: below 0.02, between 0.02 and 0.05, between 0.05 and 0.1, and higher than 0.1 amol/μg total RNA. The open circles represent the trough concentrations of tacrolimus a day before the initiation of the increase in transaminase levels. The tacrolimus concentrations in 10 patients who experienced ACR were below 5 ng/ml immediately before the increase in transaminase levels. The patients who experienced ACR after postoperative day 10 exhibited a blood tacrolimus concentration profile that increased rapidly a few days after the operation and decreased by nearly 5 ng/ml preceding the occurrence of ACR. Although the trough concentrations of tacrolimus were higher than 10 ng/ml, episodes of ACR occurred in three patients.

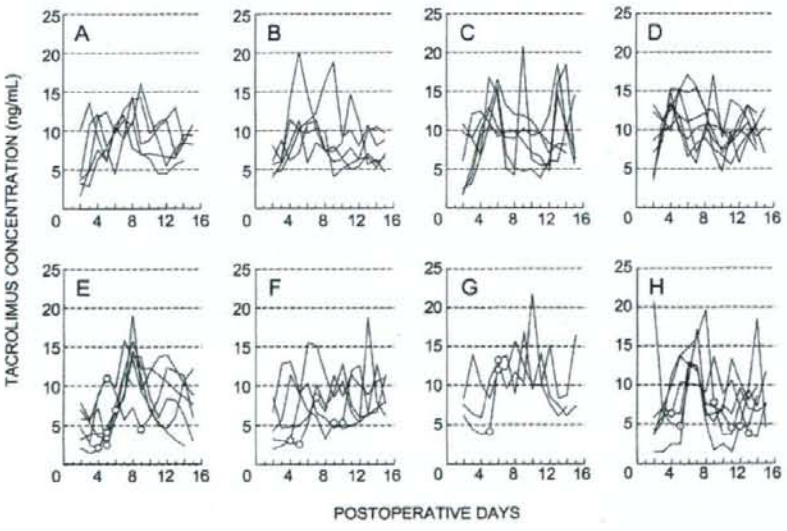

Fig. 2. The tacrolimus trough concentrations in the living-donor liver transplant patients who experienced ACR (E–H) and the event-free patients (A–D) during the15-day postoperative period. The patients were categorized into the following four groups by their MDR1 mRNA level in blood cells at postoperative day 3: below 0.02 amol/μg total RNA (A and E), between 0.02 and 0.05 amol/μg total RNA (B and F), between 0.05 and 0.10 amol/μg total RNA (C and G), and higher than 0.10 (D and H) amol/μg total RNA. The circles in E to H represent the tacrolimus trough concentrations on the day before the initiation of ACR.

To obtain additional information on the association of the MDR1 mRNA expression level in the peripheral blood cells with the tacrolimus blood concentration required to avoid ACR, a χ^2 test was carried out, and the odds ratio was calculated. After step-by-step examinations, we determined the cut-off value of 9 ng/ml of the average tacrolimus blood concentration during 15 postoperative days for subsequent statistical analyses. As shown in Table 2, the significance of a minimum tacrolimus blood concentration of 9 ng/ml during the 15-day postoperative period was found in 44 patients in this study ($P = 0.0004$). Next, we examined the effect of the MDR1 mRNA expression level on the frequency of ACR after classifying the patients based on the median expression level (0.05 amol/μg total RNA). The importance of tacrolimus blood concentration was observed in patients with a higher as well as lower MDR1 mRNA expression level in blood cells (higher MDR1, $n = 22$, $P = 0.0112$; lower MDR1, $n = 22$, $P = 0.01543$). The odds ratio revealed that the average trough level of tacrolimus of <9 ng/ml was a significant risk factor for ACR, particularly in patients with a high level of MDR1 mRNA expression in the blood cells (Table 2).

Correlation between the MDR1 mRNA Expression Level in Blood Cells and the Average Trough Levels of Tacrolimus during the 15-Day Postoperative Period. To examine whether the MDR1 mRNA expression level at postoperative day 3 was associated with the individual target trough concentration of tacrolimus during the early phase after liver transplantation, the relationship between the molecular data and the average trough concentration of tacrolimus during the 15-day postoperative period was investigated in the event-free and ACR patients. Because high-dose steroid injection treatment (pulse therapy) is a strong immunosuppressive treatment against ACR, the data of trough concentration of tacrolimus with regard to this treatment were excluded in the patients who experienced ACR. Although the data were logarithmically transformed, the observed blood MDR1 mRNA expression level was weak, but it significantly correlated with the average trough concentration of tacrolimus in both event-free patients ($r = 0.5406$, $P = 0.0077$; Fig. 3A) and ACR patients ($r = 0.4772$, $P = 0.0284$; Fig. 3B). The average trough level of tacrolimus was higher in the event-free patients than in the ACR patients ($P = 0.0008$; Fig. 3C).

Comparison of [^{14}C]Tacrolimus Accumulation in the PBMCs of *mdr1a/1b* Knockout Mice and Wild-Type Mice. The pharmacological significance of blood MDR1 on the cellular accumulation of tacrolimus was investigated using the PBMCs derived from wild-type or *mdr1a/1b* knockout mice. After the incubation of whole blood samples with [^{14}C]tacrolimus, its accumulation in the PBMCs was examined. As shown in Fig. 4, the [^{14}C]tacrolimus concentration in the PBMCs of the *mdr1a/1b* knockout mice was 2-fold higher than that in the PBMCs of the wild-type mice ($P = 0.0182$).

Discussion

MDR1 is expressed in various types of PBMCs, and it plays a role in the extrusion of substrates in these cells; therefore, MDR1 has been considered a factor that determines the intracellular concentration of drugs, including immunosuppressants, targeting blood cells. A possible correlation between MDR1 expression in peripheral blood and an ACR episode was determined by immunocytochemical analyses of patients who had undergone a heart and lung transplantation (Kemnitz et al., 1991; Yousem et al., 1993). However, in the case of kidney transplantation, it was reported that MDR1 expression could not predict the occurrence of rejection (Melk et al., 1999). In these studies, cyclosporine was used as the primary immunosuppressant. Grudé et al. (2002) reported that in liver transplant recipients who were administered tacrolimus and cyclosporine, the MDR1 expression level in the total peripheral blood was higher before ACR ($P = 0.054$); furthermore, this value was significantly lower in patients with severe infection ($P = 0.030$) (Grudé et al., 2002). These reports did not provide detailed information regarding the blood concentration of immunosuppressants; therefore, it was difficult to establish a direct relationship between these events and the expression level of MDR1 in blood cells. However, these reports suggested a possible relationship between MDR1 expression in blood cells and the susceptibility to calcineurin inhibitors.

We have reported that intestinal MDR1 prevented the intracellular accumulation of orally administered tacrolimus (Goto et al., 2003; Masuda et al., 2003, 2005; Omae et al., 2005). In addition, the intestinal MDR1 mRNA level showed a distinct inverse correlation with the concentration/dose ratio of tacrolimus immediately after liver transplantation (Hashida et al., 2001; Masuda et al., 2006). MDR1 is also expressed in the plasma membrane of peripheral leukocytes,

TABLE 2
Effect of tacrolimus trough level and MDR1 expression on the occurrence of acute cellular rejection

	Control (Event-Free) No.	Acute Cellular Rejection No.	χ^2 Test P Value	Odds Ratio (95% confidence interval)
All patients				
>9 ng/ml[a]	14	2	0.0004	14.77
<9 ng/ml	9	19		(2.752–79.33)
Patients with blood MDR1 mRNA levels below 0.05 amol/μg total RNA[b]				
>9 ng/ml	4	0	0.0154	N.A.
<9 ng/ml	6	12		
Patients with blood MDR1 mRNA levels higher than 0.05 amol/μg total RNA				
>9 ng/ml	10	2	0.0112	11.66
<9 ng/ml	3	7		(1.527–89.12)

N.A., not available.
[a] The patients were categorized by the average trough concentration of tacrolimus during the 15-day postoperative period. The trough concentrations of tacrolimus during high-dose steroid pulse therapy were excluded.
[b] The patients were categorized by the median value of the MDR1 mRNA expression level in blood cells on postoperative day 3.

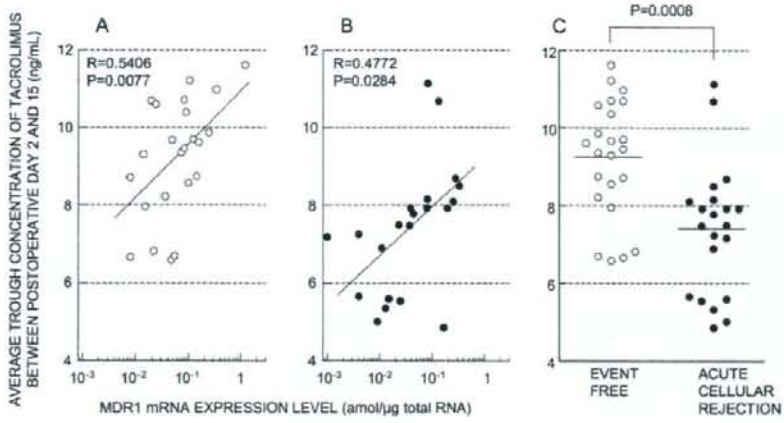

Fig. 3. Average trough concentration of tacrolimus as a function of the mRNA expression level of MDR1 in the blood cells. The average trough concentration of tacrolimus during the 15-day postoperative period is compared with the logarithmically transformed data representing the mRNA MDR1 levels in the blood cells at postoperative day 3 in the event-free patients (A) and in those who experienced acute cellular rejection (B). The average concentration of tacrolimus in the 15-day postoperative period is compared between the event-free patients and patients who experienced acute cellular rejection (C). Statistical analysis was performed using the Mann-Whitney *U* test.

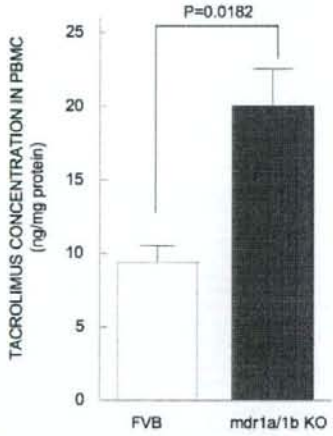

Fig. 4. Accumulation of [^{14}C]tacrolimus in PBMCs of wild-type or *mdr1a/1b* knockout mice. After drawing whole blood from the aorta of FVB-control wild-type mice or *mdr1a/1b* knockout mice, [^{14}C]tacrolimus was spiked in the blood to a final concentration of 10.4 μg/ml (6.4 kBq/ml) and incubated at 37°C for 30 min with gentle shaking. At the end of the incubation, the PBMCs were isolated, dissolved, and subjected to liquid scintillation counting, as described under *Materials and Methods*. Each column represents the mean ± S.D. of seven to eight mice. Statistical analysis was performed using the unpaired *t* test.

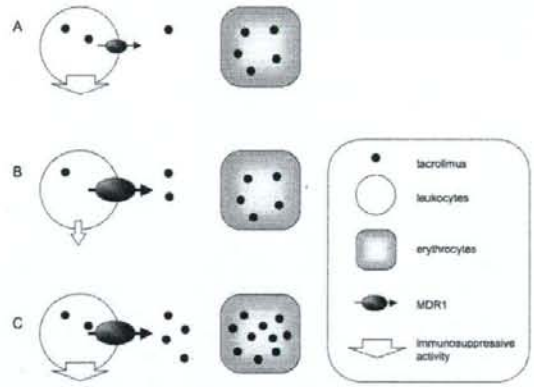

Fig. 5. Association between the MDR1 expression level and the tacrolimus concentration in leukocytes and whole blood. The scheme of the hypothesis of the present study is summarized. Most of the tacrolimus in the whole blood is distributed in the erythrocytes (Masuda and Inui, 2006), and the remaining in the plasma and leukocytes. The leukocytic tacrolimus concentration is sufficient for immunosuppression (large arrow) when MDR1 expression levels are low (A). However, with the same concentration of tacrolimus in the whole blood, the leukocytic level of tacrolimus is decreased to a level that is insufficient for immunosuppression (small arrow) when the MDR1 expression levels are high (B). Therefore, in the case of high expression levels of MDR1, a higher concentration of tacrolimus in the whole blood is required to increase its leukocytic level and enable subsequent sufficient immunosuppression (C).

mediating the cellular efflux of numerous drugs, including immunosuppressants and anticancer agents (Chaudhary et al., 1992; Klimecki et al., 1994; Ford et al., 2003). Oselin et al. (2003) quantified the MDR1 mRNA levels in several types of peripheral blood cells, but the expression levels were not affected by single nucleotide polymorphisms (G2677T and C3435T) of the *MDR1* gene. Based on this information, we hypothesized that a high expression level of MDR1 in the peripheral blood cells lowers the intracellular concentration of tacrolimus even when the concentration of the drug in the whole blood is sufficient, thereby decreasing its immunosuppressive activity in transplant patients (Fig. 5).

In the present study, we retrospectively examined the effect of MDR1 in the peripheral blood cells on the individualized target concentration of tacrolimus by analyzing the event of ACR as an endpoint in the pediatric LDLT patients. There was a markedly wide interindividual variation in the MDR1 expression level in the peripheral blood cells (Fig. 1A); however, the intraindividual variation was low and not statistically significant (Fig. 1B). Although a low concentration of tacrolimus was considered related to the occurrence of ACR, some patients who were administered a relatively high level of tacrolimus also experienced ACR (Fig. 2). Using in vitro sampled whole blood, tacrolimus was found to be mainly distributed in erythrocytes (95–98%) in dog, monkey, and human (Nagase et al., 1994). In addition, the percentage of [^{3}H]dihydro-tacrolimus associated with the lymphocytes of stable liver transplant patients (0.8% of whole blood) was significantly higher than that of patients experiencing rejec-

tion (0.3%; $P = 0.012$) (Zahir et al., 2004). Based on these findings, the leukocytic concentration rather than the whole blood concentration of tacrolimus is suggested to be a potent factor affecting the immunosuppressive activity of the drug, and leukocytic MDR1 can be a candidate molecule to decrease the cellular accumulation of tacrolimus (Fig. 5). Comparison of the MDR1 mRNA expression level with the trough concentration of tacrolimus suggested that the target concentration of tacrolimus was higher in patients with a high level of MDR1 mRNA expression in the peripheral blood cells (Fig. 3). Using the *mdr1a/1b* knockout mice, it was revealed that the blood MDR1 acted as a barrier for the cellular accumulation of tacrolimus (Fig. 4). Because of the radioactivity of [^{14}C]tacrolimus, there was a limited decrease in the concentration of tacrolimus, and saturation probably occurred. Therefore, the decrease in the concentration of tacrolimus, which is comparable with the clinical situation, may be more affected by the MDR1 expression in the blood cells. These findings suggest that the MDR1 expression level in the peripheral blood cells is a potential pharmacological marker of tacrolimus concentration and that it can be used to establish the individualized target concentration of this drug.

Therapeutic drug monitoring contributes to the development of individualized pharmacotherapy in patients administered toxic agents, including calcineurin inhibitors. The general therapeutic window of tacrolimus ranges between 5 and 20 ng/ml in transplant patients (Venkataramanan et al., 1995; Masuda and Inui, 2006; Oellerich and Armstrong, 2006). However, the target range always varies with the duration of the postoperative period and the patient status. In patients undergoing liver transplantation, the target tacrolimus concentration is between 10 and 20 ng/ml during the 15-day postoperative period. However, the actual level of tacrolimus is relatively low because of its severe adverse effects. We previously reported that at least 7 ng/ml of the average trough concentration of tacrolimus is required immediately after transplantation to prevent ACR (Masuda et al., 2006). However, the ACR episode occurred in 22% of patients in whom the blood tacrolimus concentration was maintained at more than 7 ng/ml. Because tacrolimus targets the leukocytes, the information regarding the concentration of tacrolimus in the whole blood is thought to be insufficient to control the ACR episode in some patients. Therefore, an additional biological marker reflecting the leukocytic concentration of tacrolimus should be identified to establish an individualized target concentration of the drug. In the present study, we have found that a higher target concentration of tacrolimus is required in patients with a high expression level of MDR1 mRNA (>0.05 amol/μg total RNA) than in those with a low expression level (Table 2; Fig. 3). If the expression level of MDR1 mRNA in the peripheral blood is examined immediately after surgery, we can focus on patients with a high risk of ACR and maintain them on a high level (at least 9 ng/ml) of tacrolimus during the 15-day postoperative period.

In conclusion, the MDR1 mRNA expression level in the peripheral blood is a possible predictor of the susceptibility to tacrolimus; a high expression level of MDR1 might cause ACR, necessitating a higher blood concentration of tacrolimus (Fig. 5). Although further analyses with or without intervention should be investigated to establish the clinical significance of blood MDR1 as a pharmacological marker, molecular information about MDR1 expression in peripheral blood cells may be useful in the establishment of an individualized target concentration of tacrolimus in children after liver transplantation.

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JPET PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS aspet

Impact of MDR1 and CYP3A5 on the oral clearance of tacrolimus and tacrolimus-related renal dysfunction in adult living-donor liver transplant patients

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Objective **The potential influence of the multidrug resistance 1 (*MDR1*) gene and the cytochrome P450 (CYP) genes, *CYP3A4* and *CYP3A5*, on the oral clearance (CL/F) of tacrolimus in adult living-donor liver transplant patients was examined. Furthermore, the development of renal dysfunction was analyzed in relation to the *CYP3A5* genotype.**

Methods **Sixty *de novo* adult liver transplant patients receiving tacrolimus were enrolled in this study. The effects of various covariates (including intestinal and hepatic mRNA levels of MDR1 and CYP3A4, measured in each tissue taken at the time of transplantation, and the *CYP3A5*3* polymorphism) on CL/F during the first 50 days after surgery were investigated with the nonlinear mixed-effects modeling program.**

Results **CL/F increased linearly until postoperative day 14, and thereafter reached a steady state. The initial CL/F immediately after liver transplantation was significantly affected by the intestinal MDR1 mRNA level ($P<0.005$). Furthermore, patients carrying the *CYP3A5*1* allele in the native intestine, but not in the graft liver, showed a 1.47 times higher (95% confidence interval, 1.17–1.77 times, $P<0.005$) recovery of CL/F with time than patients having the intestinal *CYP3A5*3/*3* genotype. The cumulative incidence of renal dysfunction within 1 year after transplantation, evaluated by the Kaplan–Meier method, was significantly associated with the recipient's but not donor's *CYP3A5* genotype (**1/*1* and **1/*3* vs. **3/*3*: recipient, 17 vs. 46%, $P<0.05$; donor, 35 vs. 38%, $P=0.81$).**

Conclusion **These findings suggest that the *CYP3A5*1* genotype as well as the MDR1 mRNA level in enterocytes contributes to interindividual variation in the CL/F of tacrolimus in adult recipients early after living-donor liver transplantation. Furthermore, CYP3A5 in the kidney may play a protective role in the development of tacrolimus-related nephrotoxicity. *Pharmacogenetics and Genomics* 18:413–423 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

Pharmacogenetics and Genomics 2008, 18:413–423

Keywords: CYP3A5, living-donor liver transplantation, multidrug resistance 1, nephrotoxicity, pharmacogenomics, pharmacokinetics, tacrolimus

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Received 14 July 2007 **Accepted** 23 January 2008

Introduction

Initially, living-donor liver transplantation (LDLT) was adapted mainly to children with end-stage liver diseases, such as biliary atresia and hepatoblastoma [1]. At present, the technique is used as a treatment option for adult patients with viral hepatic cirrhosis or hepatocellular carcinoma, etc. [2].

Tacrolimus, an immunosuppressant widely used in liver transplantation, has a narrow therapeutic window (5–15 ng/ml) and shows considerable interindividual and intraindividual variability in clinical pharmacokinetics [3,4]. Therefore, therapeutic drug monitoring (TDM) is needed to individualize the tacrolimus dosage for the prevention of allograft rejection as well as adverse effects such as nephrotoxicity [5,6]. Tacrolimus is primarily metabolized by hepatic cytochrome P450 (CYP) 3A4 and 3A5 with the majority of the metabolites eliminated into bile [7]. P-glycoprotein, which is a product of the multidrug resistance 1 (*MDR1/ABCB1*) gene, plays an important role in limiting the oral absorption of immunosuppressive drugs [7]. We clarified that the intestinal MDR1 mRNA affects the postoperative trough levels of tacrolimus in LDLT recipients [8,9]. We have also demonstrated that the *CYP3A5*1* allele in the native intestine as well as the graft liver is associated with the reduced concentration/dose (C/D) ratio of tacrolimus in LDLT recipients [10,11].

Population pharmacokinetic analysis with the nonlinear mixed-effects modeling (NONMEM) program has been widely used to identify the covariates that significantly

influence the pharmacokinetics of tacrolimus in liver transplant patients [12–17]. We previously developed population pharmacokinetic models for tacrolimus with NONMEM in pediatric and adult patients receiving LDLT, and revealed that the clearance of tacrolimus increased with time after surgery [18,19]. In our recent study, the intestinal expression level of MDR1 mRNA was shown to have a significant effect on the initial value of oral clearance (CL/F) of tacrolimus in pediatric LDLT recipients [20]. Furthermore, the recovery of CL/F with time was demonstrated to be twice higher in patients receiving a *CYP3A5*1*-carrying graft liver (CYP3A5 expressors) than in patients with the hepatic *CYP3A5*3/*3* genotype (CYP3A5 nonexpressors) [20]. The graft size in adult LDLT recipients is inevitably smaller than the ideal liver size for the recipient's metabolic demands, whereas the graft liver in most pediatric LDLT recipients is large enough [21,22]. A small-for-size graft, in which the functional liver mass is reduced and necessary for liver regeneration, is considered as an important factor affecting the disposition of drugs including tacrolimus in adult liver transplant patients [17,23,24]. Therefore, it is likely that the major organ(s) and molecule(s) responsible for the pharmacokinetic variability of tacrolimus differ between pediatric and adult LDLT recipients, owing to the difference in size of the grafted liver.

Renal dysfunction is a frequent complication in transplant patients receiving the calcineurin inhibitors tacrolimus and cyclosporine [25]. Although there are clinical data suggesting that renal P-glycoprotein is a risk factor for nephrotoxicity of calcineurin inhibitors, conflicting results have been reported with regard to a causal relationship between the *MDR1* G2677T/A and C3435T genetic polymorphisms and nephrotoxicity [26,27]. In contrast, CYP3A5 is also expressed in the kidney, and the formation of cyclosporine and tacrolimus metabolites by renal CYP3A5 *in vitro* has been demonstrated [28,29]. Recently, decreased expression of renal CYP3A5 was reported in patients with calcineurin inhibitor-related nephrotoxicity [30]. Therefore, it can be hypothesized that CYP3A5 in the kidney has an influence on the development of nephrotoxicity induced by calcineurin inhibitors in transplant patients, probably by reducing drug accumulation within renal cells.

In this study, the impact of hepatic or intestinal *MDR1*, *CYP3A4*, and *CYP3A5* genes on the CL/F of tacrolimus in *de novo* adult LDLT recipients was examined by using a population pharmacokinetic approach. Furthermore, the development of renal dysfunction within 1 year after transplantation was analyzed in relation to the donor's and recipient's *CYP3A5* genotypes.

Methods

Patients and ethics

A total of 60 *de novo* Japanese adult liver transplant patients receiving tacrolimus were enrolled in this study. All patients underwent LDLT between May 2005 and March 2006 at the Department of Surgery, Kyoto University Hospital, Kyoto, Japan. Patients were eligible for the study if pharmacogenomic data for both donors and recipients were available. Patients treated with the concomitant use of potent inducers or inhibitors of CYP3A and P-glycoprotein were excluded, but patients receiving intravenous high-dose corticosteroids for the treatment of a subclinical rejection episode were not excluded. In addition, the use of low-dose fluconazole for prophylaxis of fungal infections was allowed. This study was conducted in accordance with the Declaration of Helsinki and its amendments, and the protocol was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine. Written informed consent was obtained from each patient.

Immunosuppressive therapy

After liver transplantation, we started tacrolimus-based immunosuppressive therapy combined with corticosteroids. Tacrolimus (Prograf, Astellas Pharma Inc., Tokyo, Japan) was orally administered at 0.05 mg/kg/day twice daily (at 09:00 and 21:00 h). The dosage was adjusted to achieve the target trough level, which was set at between 10 and 15 ng/ml during the first 2 weeks, at approximately 10 ng/ml thereafter, and at between 5 and 10 ng/ml starting in the second month. Methylprednisolone (10 mg/kg) was intravenously administered at the time of graft reperfusion, and then the dosage was gradually reduced, and the patients were switched to oral prednisolone 1 week after surgery. The dosage was gradually tapered off and discontinued between 3 and 6 months after the transplant if clinically possible.

Tacrolimus assay

Blood samples for the routine TDM of tacrolimus trough levels were collected into ethylenediaminetetra-acetic acid-containing tubes before the morning dose (at 08:00 h) starting on postoperative day 2. The concentration of tacrolimus in whole blood was determined by a microparticle enzyme immunoassay method using an IMx analyzer (Abbott Japan, Tokyo, Japan) on the day of blood collection [31]. Clinical laboratory test markers were simultaneously measured in the morning during hospitalization.

Pharmacogenomic analysis

Tissue samples (approximately 1 mm^3) of the upper jejunum and liver were obtained from part of the Roux-en-Y limb for biliary reconstruction and the biopsy specimens for histopathological examination of the graft liver at surgery, respectively.

For the *MDR1* polymorphisms, we previously clarified that the G2677T/A and C3435T polymorphisms did not influence the mRNA expression level in enterocytes or

the C/D ratio of tacrolimus in LDLT recipients [32]. Regarding the *CYP3A4* mutant allele, the *CYP3A4*1B* allele is very rare in Asians and seems not to be responsible for the variability in the disposition of cyclosporine [33]. In contrast, the *CYP3A5*3* allele resulting in a functional CYP3A5 deficiency was reported as the major mutant allele in the Japanese population [34,35]. Therefore, in this study we genotyped the *CYP3A5*3* polymorphism as described previously [11]. Briefly, genomic DNA was extracted from a homogenate of biopsy specimens of the graft liver and the intestinal mucosa or peripheral blood of recipients with MagNA Pure LC DNA Isolation kit I (Roche, Mannheim, Germany). The *CYP3A5*3* polymorphism was determined by the polymerase chain reaction (PCR)-restriction fragment length polymorphism method.

Total RNA was extracted from the homogenate from the graft liver and the intestinal mucosa with MagNA Pure LC RNA Isolation kit II (Roche) and reversed transcribed as described earlier [9]. Subsequently, the expression of MDR1, CYP3A4, and CYP3A5 mRNAs was quantified by real-time PCR using an ABI Prism 7700 Sequence Detector (Applied Biosystems, Foster, California, USA) according to a procedure described previously [10].

Population pharmacokinetic analysis

A total of 1827 blood samples collected within the first 50 days after LDLT were used for the population pharmacokinetic analysis of tacrolimus with the program NONMEM (double precision NONMEM version V level 2.1, PREDPP version III level 1.1, and NMTRAN version II level 2.1) using the first-order conditional estimation method [36]. As blood samples were collected predose in this study, the pharmacokinetics of tacrolimus after oral administration was described by a 1-compartment model with first-order elimination, and the basic pharmacokinetic parameters estimated were CL/F (l/h) and apparent volume of distribution (V/F, l). For the random error model, the interindividual variability of pharmacokinetic parameters and the residual variability between the observed and predicted concentrations were best described with an exponential error model and an additive error model, respectively.

In the model development process, a forward inclusion and backward elimination approach was used to build a regression model for CL/F. Owing to a lack of information on the distribution phase during the trough level monitoring, we did not perform a covariate analysis for the V/F of tacrolimus ($V/F = \theta_2$). The effect of a covariate was assessed with a χ^2 test of the difference in the objective function values (OFV) calculated by NONMEM between the models with and without a covariate. If a difference in the OFV between two models [−2 log likelihood difference (−2 LLD)] was more than 3.84 ($P < 0.05$) with 1 degree of freedom on the inclusion of each covariate, the covariate was considered statistically significant.

Previously, tacrolimus clearance was shown to increase with time after liver transplantation and reach a steady state within the first month [15,20]. According to this observation, we first examined the relationship of CL/F with time after surgery by using the following cutoff model:

$$CL/F = \theta_1 + \theta_3 \times XPOD$$

if POD < CP, XPOD = POD; otherwise, XPOD = CP where θ_1 and θ_3 are the initial value of CL/F immediately after LDLT and the increase in CL/F per day, respectively; POD is postoperative days and XPOD is an arbitrary value of POD; and CP is the cutoff point for POD.

The influence of patient characteristics including demographics and clinical laboratory data on CL/F in the derived time-dependent clearance model was next examined using an exponential function, with the effect of each covariate normalized by the respective median value. At each step in the selection process, the factor showing the largest −2 LLD among significant covariates was incorporated into the cutoff model. Then, all factors that were found to be significant were reassessed, and the factor that showed the largest −2 LLD was included in the intermediate model. This step was repeated until the greatest −2 LLD was below 3.84 to obtain the full model. Throughout the covariate analysis, the precision of the parameter estimate [95% confidence interval (CI)] and the reduction in interindividual and residual variability as well as scatter plots of observed versus model-predicted concentrations were used as another indicator of the goodness-of-fit in each model. Upon the independent deletion of each covariate, a −2 LLD between the full and reduced models of more than 7.88 ($P < 0.005$) was required to retain a covariate in the final population model (model 1).

Finally, we investigated all possible relationships of CL/F in model 1 with hepatic or intestinal MDR1 and CYP3A4 mRNAs and the *CYP3A5*1* allele, by using both multiplicative and additive models. Quantitative covariates of MDR1 and CYP3A4 mRNAs were transformed into dichotomous variables (high or low) according to the median value. As the *CYP3A5* genotype can be a relevant predictor for CYP3A5 expression, the impact of the *CYP3A5*1* allele on CL/F was examined as a binary variable [expressors (**1/*1* and **1/*3*) or nonexpressors (**3/*3*)] for the CYP3A5 enzyme. In this analysis, we estimated the fractional increase in CL/F associated with high mRNA levels of MDR1 and CYP3A4 or the functional expression of CYP3A5. The statistical significance of pharmacogenomic data as a covariate for CL/F

was tested according to the approach described above to develop the final population model (model 2).

Analysis of renal dysfunction in relation to the *CYP3A5* genotype

Tacrolimus-related nephrotoxicity was defined as an initial increase in the serum creatinine (SCR) level greater than 0.5 mg/dl above the pretransplant baseline. To avoid the potential influence of preoperative renal dysfunction on the incidence of adverse events, we excluded three patients who had renal dialysis ($n = 1$) and were diagnosed with renal impairment ($n = 2$) before LDLT. A 1-year follow-up after the transplant was performed to investigate the possible association between renal dysfunction and the *CYP3A5*1* status of donors as well as recipients. The medical records of each patient were reviewed for abstraction of SCR values, which had been reported on the day of regular hospital visits (3, 6, 9, and 12 months after transplantation) for outpatients, as well as throughout the hospitalization for inpatients. Data available near the intended time points were used to calculate the average values for dose requirements and trough blood concentrations of tacrolimus.

Statistical analysis

The statistical significance of differences in parametric and nonparametric values between two groups was analyzed with the unpaired *t*-test and the Mann–Whitney *U*-test, respectively. Hardy–Weinberg equilibrium for genotype frequency was examined by the χ^2 test. The proportion of patients with renal dysfunction was determined with the Kaplan–Meier method, and the log-rank test was used to examine the significance of differences between two curves. *P* value of less than 0.05 was considered statistically significant.

Results

Patients and pharmacogenomic data

The demographics and clinical laboratory data for the study population are shown in Table 1. The most common primary disease leading to LDLT was cirrhosis (56/60, 93.3%). The median value of the graft-to-recipient weight ratio (GRWR) was significantly lower than in our earlier study of 130 pediatric LDLT recipients [20] (1.1 vs. 2.6%, $P < 0.05$, Mann–Whitney *U*-test). Although most of the blood tacrolimus concentrations were maintained within the therapeutic window (5–15 ng/ml), the trough levels were variable at around the target range, especially during the first week after transplantation (Fig. 1).

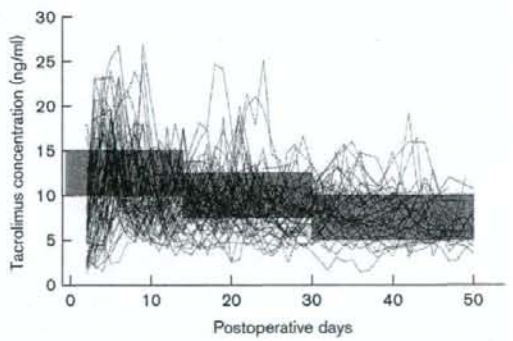
The pharmacogenomic data for this study population are summarized in Table 2. The expression profiles of MDR1 and CYP3A4 mRNAs in the native intestine as well as the graft liver were generally comparable with those reported previously for pediatric cases of LDLT [20]. The CYP3A5 mRNA content was significantly correlated with the *CYP3A5* genotype in both tissues ($P < 0.05$). The median mRNA level of CYP3A5 in the native intestine was similar to that in pediatric LDLT recipients [20] (0.24 vs. 0.11 amol/μg total RNA, respectively). The median

Table 1 Characteristics of the study population ($n = 60$)

Demographics	
Sex (male/female)	29/31
Age (year)	55 (29–70)
Body weight (kg)	57 (41–85)
Graft weight (g)	695 (425–935)
Graft-to-recipient weight ratio (%)	1.1 (0.7–2.1)
Primary disease	
Cirrhosis	56
Hepatitis C virus infection	24
Hepatitis B virus infection	19
Primary biliary cirrhosis	6
Alcoholic cirrhosis	3
Unknown cirrhosis	4
Others	4
Donor (male/female)	32/28
Donor age (year)	47 (21–66)
Clinical laboratory data[a]	
Hematocrit (%) (33.7–49.8)	28.0 (19.8–45.9)
Total protein (g/dl) (6.3–8.1)	6.6 (4.6–8.5)
Serum albumin (g/dl) (3.9–5.1)	3.1 (2.2–4.4)
Total bilirubin (mg/dl) (0.3–1.3)	2.9 (0.4–38.1)
AST (IU/l) (13–33)	51 (23–173)
ALT (IU/l) (8–42)	29 (7–129)
γ-Glutamyl transpeptidase (IU/l) (9–54)	36 (11–873)
Serum creatinine (mg/dl) (0.6–1.1)	0.8 (0.3–1.9)
Blood urea nitrogen (mg/dl) (8–22)	16 (4–82)
Pharmacokinetic data of tacrolimus[b]	
Number of samples	1827
Number of samples per patient	30 (13–49)
Dose (mg/day)	3.0 (0.2–16.0)
Trough concentration (ng/ml)	8.6 (1.6–26.9)

Data are expressed as a number or median (range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDLT, living-donor liver transplantation.
[a]Range in parentheses indicates reference data at Kyoto University Hospital, Kyoto, Japan, for normal adult men.
[b]Dose and trough concentration are based on all data from the first 50 days after LDLT.

Fig. 1



Tacrolimus trough concentrations in whole blood vs. postoperative days for all recipients ($n = 60$). Data from the same patient are connected with a line. The closed area shows the target range in each postoperative period (10–15 ng/ml during the first 2 weeks, approximately 10 ng/ml thereafter, and 5–10 ng/ml starting in the second month).

Table 2 Pharmacogenomic data for the study population ($n=60$)

	Recipient	Donor
	Native intestine	Graft liver
Expression level		
MDR1 mRNA (amol/μg total RNA)	0.15 (0.01–1.1)	0.81 (0.40–2.9)
CYP3A4 mRNA (amol/μg total RNA)	3.5 (0.001–16)	50 (11–130)
CYP3A5 mRNA (amol/μg total RNA)	0.24 (0.001–2.5)	1.6 (0.26–23)
**1/*1* and **1/*3* (amol/μg total RNA)	0.73[a] (0.001–2.5)	8.4[a] (3.9–23)
**3/*3* (amol/μg total RNA)	0.16 (0.001–1.5)	1.1 (0.26–6.4)
Genotype frequency[b]		
*CYP3A5*1/*1*	3 (5.0%)	1 (1.7%)
*CYP3A5*1/*3*	17 (28.3%)	23 (38.3%)
*CYP3A5*3/*3*	40 (66.7%)	36 (60.0%)

Data are expressed as the median (range) for expression level and as the number of patients (percent) for genotype frequency.
[a] $P<0.05$, significant difference in the distribution from the *CYP3A5*3/*3* group (Mann–Whitney *U*-test).
[b] Genotype frequency was not significantly different from that predicted by Hardy–Weinberg equilibrium ($P>0.05$ for recipient and donor, χ^2 test).

(range) expression level of CYP3A5 mRNA in the graft liver was 1.6 (0.26–23) amol/μg total RNA, which was also comparable with that in pediatric LDLT recipients [20] [1.0 (0.10–10) amol/μg total RNA]. The genotype frequencies of the *CYP3A5*3* polymorphism for both donors and recipients were in Hardy–Weinberg equilibrium, and well consistent with the earlier report for pediatric cases of LDLT [20].

Development of a time-dependent clearance model for tacrolimus

The OFV of the basic model without any covariate was 7200.22. Initially, we tested allometric scaling of CL/F and V/F in the basic model by using body weight. The inclusion of body weight, however, did not significantly decrease the OFV. In addition, neither age nor sex showed any correlation with the individual Bayesian estimates for CL/F and V/F, which were obtained using the post-hoc option of NONMEM. Therefore, the basic model was used for the subsequent analysis.

In the plot of weighted residuals against postoperative days, the basic model systematically underestimated blood tacrolimus concentrations for the first few weeks after transplantation. To describe the postoperative recovery of CL/F after LDLT, the factor of postoperative days was adopted as a covariate for the cutoff model. We examined three different cutoff points for postoperative days (7, 14, and 21) and confirmed that the best fit was obtained using 14 (OFV = 6079.49). Accordingly, a time-dependent clearance model for tacrolimus was developed, where CL/F increased linearly after LDLT but did not change with time after postoperative day 14.

Influence of patient characteristics on tacrolimus CL/F

We investigated the potential influence of the graft liver (GRWR) and clinical laboratory data on CL/F in the derived cutoff model. In the first selection step, GRWR did not significantly reduce the OFV (−2 LLD = 1.00), whereas the effect of SCR showed the largest improvement of fit (−2 LLD = 57.61) and was incorporated into the cutoff model. The effects of total bilirubin (TBIL) and serum albumin (ALB) were included in the intermediate model in the second and third selection steps, respectively. In the fourth selection step, a significant improvement of fit was no longer observed to obtain the full model. No covariate was excluded by backward elimination to derive the following final population model for CL/F (model 1):

$$\mathrm{CL/F} = (0.340 + 0.964 \times \mathrm{XPOD}) \times \mathrm{EXP}(-0.423 \times \mathrm{SCR}/0.8) \times \mathrm{EXP}(-0.0399 \times \mathrm{TBIL}/2.9) \times \mathrm{EXP}(0.476 \times \mathrm{ALB}/3.1)$$

(if POD < 14, then XPOD = POD; otherwise, XPOD = 14).

Influence of pharmacogenomic data on tacrolimus CL/F

We examined the contribution of pharmacogenomic data on CL/F in model 1, using the mRNA levels of MDR1 and CYP3A4 as well as the *CYP3A5*1* genotype. As shown in Table 3, the OFV was markedly decreased by more than 10 U from model 1 in the first selection step, when including the effects of the intestinal *CYP3A5*1* allele with multiplicative and additive models (−2 LLD = 10.39 and 10.84, respectively), as well as the effect of the intestinal CYP3A4 mRNA by an additive function (−2 LLD = 10.98). We selected the multiplicative model for the intestinal *CYP3A5*1* allele, which had the smallest interindividual variability of CL/F ($\omega_{CL/F} = 47.2\%$). In the second selection step, the influence of the mRNA levels of MDR1 and CYP3A4 in the native intestine improved likewise the fit of the intermediate model (−2 LLD = 9.50 and 9.74, respectively). We chose the effect of the intestinal MDR1 mRNA, showing the smaller interindividual variability of CL/F compared with that of CYP3A4 ($\omega_{CL/F} = 46.2$ vs. 48.1%, respectively). In the third selection step, the effect of the hepatic *CYP3A5*1* allele was found to be significant (−2 LLD = 5.56). This covariate, however, was excluded by backward elimination and the final population model (model 2, Table 4) was developed. Although the residual variability and the interindividual variability of V/F did not decrease in model 2 compared with model 1, the interindividual variability of CL/F could be lowered to 46.2% in model 2 from 51.8% in model 1 (Table 4). Although the scatter plot of population prediction with model 2 vs. observed concentration showed a marked deviation (Fig. 2a), the Bayesian method using individual blood concentration data provided more accurate and less biased predictions of blood tacrolimus concentrations (Fig. 2b).

Figure 3 shows that the population mean predictions of CL/F based on the final model 2 in a typical patient having the median values of SCR, TBIL, and ALB. The increase in CL/F with postoperative days was 1.47 times higher (95% CI, 1.17–1.77 times, $P < 0.005$) in patients

Table 3 Summary of model building process for tacrolimus CL/F using pharmacogenomic data

Models[a]	Covariates[b]	−2 LLD[c] First selection	−2 LLD[c] Second selection	−2 LLD[c] Third selection
Multiplicative model $CL/F=(\theta_1+\theta_3\times XPOD)\times EXP(-\theta_4\times SCR/0.8)\times EXP(-\theta_5\times TBIL/2.9)\times EXP(\theta_6\times ALB/3.1)\times\theta_7^{FLAG}$	Donor/graft liver			
	MDR1 mRNA	0.15 (51.7%)		
	CYP3A4 mRNA	0.58 (51.5%)		
	*CYP3A5*1* allele	4.84 (49.6%)[d]	4.17 (45.5%)[d]	5.56 (46.0%)*
	Recipient/native intestine			
	MDR1 mRNA	2.11 (50.9%)		
	CYP3A4 mRNA	0.46 (51.6%)		
	*CYP3A5*1* allele	10.39 (47.2%)[d,e]		
Additive model $CL/F=(\theta_1+\theta_3\times XPOD)\times EXP(-\theta_4\times SCR/0.8)\times EXP(-\theta_5\times TBIL/2.9)\times EXP(\theta_6\times ALB/3.1)+\theta_7\times FLAG$	Donor/graft liver			
	MDR1 mRNA	1.43 (51.8%)		
	CYP3A4 mRNA	0.19 (51.7%)		
	*CYP3A5*1* allele	0.01 (51.8%)		
	Recipient/native intestine			
	MDR1 mRNA	8.00 (51.1%)[d]	9.50 (46.2%)[d,e]	
	CYP3A4 mRNA	10.98 (52.2%)[d]	9.74 (48.1%)[d]	3.08 (46.5%)
	*CYP3A5*1* allele	10.84 (49.2%)[d]		

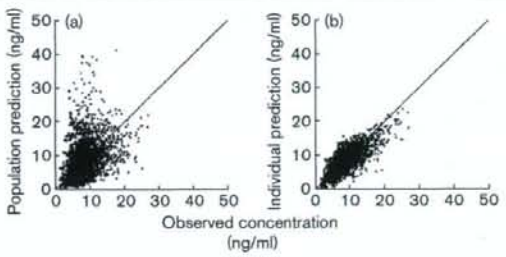
ALB, serum albumin; FLAG, indicator variable having 0 or 1; −2 LLD, −2 log likelihood difference [a reduction of the objective function value (OFV)]; POD, postoperative days; SCR, serum creatinine; TBIL, total bilirubin; XPOD, an arbitrary value of POD.
[a]Model 1 [$CL/F=(\theta_1+\theta_3\times XPOD)\times EXP(-\theta_4\times SCR/0.8)\times EXP(-\theta_5\times TBIL/2.9)\times EXP(\theta_6\times ALB/3.1)$ (if POD <14, XPOD=POD; otherwise, XPOD=14); $V/F=\theta_2$; OFV=5954.27] was used as a basic model to develop a refined population model including pharmacogenomic covariates (model 2).
[b]A high expressor of MDR1 and CYP3A4 was identified by an mRNA level greater than the respective median value (shown in Table 2). If the donor or recipient was a high expressor of MDR1 or CYP3A4 mRNA, FLAG=1; otherwise, FLAG=0. If the donor or recipient carried the *CYP3A5*1* allele, FLAG=1; otherwise, FLAG=0.
[c]Interindividual variability of CL/F is presented in parentheses beside the value of −2 LLD.
[d]$P<0.05$, significant difference in the goodness-of-fit between models with and without a covariate.
[e]The covariate showing the smallest interindividual variability of CL/F among significant covariates that yielded a close −2 LLD was incorporated into the intermediate model.

Table 4 Final population pharmacokinetic estimates for tacrolimus in adult LDLT patients

Parameters	Model 1[a]	Model 2[b]
Fixed effects		
θ_1 (l/h)	0.340 (0.0928–0.587)	–
θ_2 (l)	644 (531–757)	631 (514–748)
θ_3 (l/h/day)	0.964 (0.612–1.32)	0.784 (0.464–1.10)
θ_4	0.423 (0.257–0.599)	0.443 (0.269–0.617)
θ_5	0.0399 (0.0243–0.0555)	0.0425 (0.0272–0.0578)
θ_6	0.476 (0.196–0.756)	0.529 (0.229–0.829)
θ_7	–	1.47 (1.17–1.77)
θ_8 (l/h)	–	1.06 (0.160–1.96)
Random effects		
$\omega_{CL/F}$ (%)	51.8 (39.3–61.7)	46.2 (35.8–54.6)
$\omega_{V/F}$ (%)	66.5 (53.7–77.2)	68.0 (54.3–79.4)
σ (ng/ml)	2.73 (2.53–2.92)	2.73 (2.52–2.92)

Data are expressed as the mean value (95% CI).
[a]Model 1 was described with the following equations: $CL/F=(\theta_1+\theta_3\times XPOD)\times EXP(-\theta_4\times SCR/0.8)\times EXP(-\theta_5\times TBIL/2.9)\times EXP(\theta_6\times ALB/3.1)$ (if POD <14, then XPOD=POD; otherwise, XPOD=14) and $V/F=\theta_2$.
[b]Model 2 was described with the following equations: $CL/F=\theta_8\times MDR1+\theta_3\times XPOD\times EXP(-\theta_4\times SCR/0.8)\times EXP(-\theta_5\times TBIL/2.9)\times EXP(\theta_6\times ALB/3.1)\times\theta_7^{CYP3A5}$ (if POD <14, then XPOD=POD; otherwise, XPOD=14; if the recipient was a *CYP3A5*1* allele carrier, then CYP3A5=1; otherwise, 0; if the intestinal MDR1 mRNA level >0.15 amol/μg total RNA, then MDR1=1; otherwise, 0) and $V/F=\theta_2$.

Fig. 2

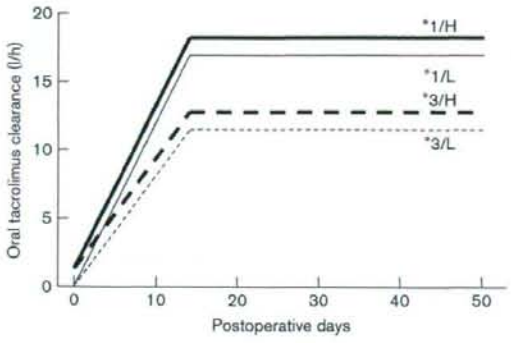


Scatter plots of observed tacrolimus concentrations vs. population predictions (a) and individual predictions by the Bayesian method with the final model 2 (b). The solid line shows the line of identity.

carrying the intestinal *CYP3A5*1* allele than in patients with the *CYP3A5*3/*3* genotype in enterocytes (Fig. 3 and Table 4). Furthermore, the higher expression of intestinal MDR1 mRNA (>0.15 amol/μg total RNA) resulted in the augmentation of CL/F by 1.06 l/h (95% CI, 0.160–1.96 l/h, $P<0.005$) (Fig. 3 and Table 4).

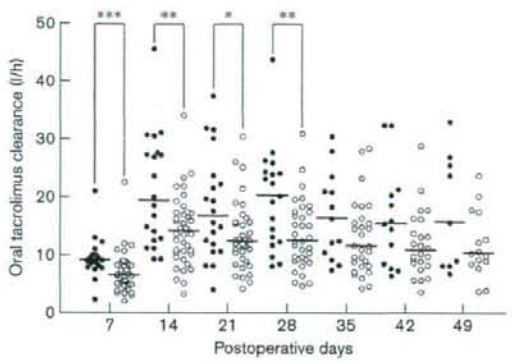
The longitudinal influence of the enterocyte *CYP3A5*1* allele on CL/F was examined by dividing the immediate postoperative period into seven different weeks. The individual CL/F estimates on the last day in each week were obtained with the Bayesian method by using blood concentration data available during the respective week and the final model 2. The significant influence of the intestinal *CYP3A5*3* polymorphism on the individual CL/F estimates was confirmed until postoperative day 28, but thereafter the distribution of the individual CL/F

Fig. 3



Time-dependent changes in population mean estimates of oral tacrolimus clearance obtained with the final model 2 during the first 50 postoperative days. The bold and thin solid lines represent the tacrolimus CL/F in a typical patient (serum creatinine, 0.8 mg/dl; total bilirubin, 2.9 mg/dl; serum albumin, 3.1 mg/dl) carrying the intestinal *CYP3A5*1* allele with high (*1/H) and low (*1/L) intestinal MDR1 mRNA levels, respectively. The bold and thin dotted lines indicate the tacrolimus CL/F in a typical patient having the intestinal *CYP3A5*3/*3* genotype with high (*3/H) and low (*3/L) intestinal MDR1 mRNA levels, respectively.

Fig. 4



The influence of intestinal *CYP3A5*1* genotype on the individual Bayesian estimates of oral tacrolimus clearance throughout the first seven weeks after LDLT. The closed and open circles represent the data from patients carrying the *CYP3A5*1* allele (CYP3A5 expressors) and patients with the *CYP3A5*3/*3* genotype (CYP3A5 nonexpressors) in the native intestine, respectively. Each bar indicates the median value. $*P<0.05$, $**P<0.01$, $***P<0.001$, significant difference between two groups (Mann–Whitney *U*-test). LDLT, living-donor liver transplantation.

estimates was not significantly different between patients with and without the intestinal *CYP3A5*1* allele (Fig. 4).

Tacrolimus exposure and nephrotoxicity according to the *CYP3A5* genotype

Table 5 shows the daily dose and exposure parameters of tacrolimus over a 1-year period after LDLT. Trough blood concentration (C_0) throughout the follow-up period did not differ between expressors (**1/*1* and **1/*3*) and nonexpressors (**3/*3*) of CYP3A5 in donors as well as recipients. On day 7, bodyweight-normalized dose and C/D ratio were significantly affected by the *CYP3A5*1* allele in both donors and recipients, whereas the *CYP3A5*1* allele in recipients alone significantly influenced these two parameters on day 14. After the first month, the *CYP3A5*1* allele in donors but not in recipients had a significant effect on tacrolimus dose and C/D ratio. These results were not altered when three patients with preoperative renal dysfunction were excluded from analysis.

The cumulative incidence of renal dysfunction within 1 year after transplantation was not affected by the *CYP3A5*1* allele in donors [**1/*1* and **1/*3* vs. **3/*3*: 8/23 (35%) vs. 13/34 (38%), $P = 0.81$] (Fig. 5a). In contrast, the *CYP3A5*3/*3* genotype in recipients was significantly associated with the increased incidence of tacrolimus-related nephrotoxicity [**1/*1* and **1/*3* vs. **3/*3*: 3/18 (17%) vs. 18/39 (46%), $P < 0.05$; hazard ratio (95% CI), 3.16 (1.01–6.16)] (Fig. 5b).

Discussion

Population pharmacokinetic and pharmacogenomic analysis with the NONMEM program has advantages in identifying statistically significant covariates among various factors as well as in determining the impact of genomic variation on the clinical pharmacokinetics of drugs [20,37–40]. Figure 1 shows that the difficulty in maintaining the blood tacrolimus concentrations within the target range despite the daily adjustment of oral dosage based on TDM, especially in the first week after LDLT. Therefore, we attempted to establish a more accurate population pharmacokinetic model for tacrolimus by using pharmacogenomic information and clinical data obtained during the initial posttransplant period. In this study, we have first clarified that the *CYP3A5*1* allele as well as the MDR1 mRNA level in enterocytes is an important determinant for the CL/F of tacrolimus in adult recipients early after LDLT.

In a time-dependent clearance model for tacrolimus, the time needed for CL/F to reach a steady state was estimated as 14 days. In pediatric LDLT recipients, the recovery of CL/F was calculated to take 21 days [20]. These results suggest that the tacrolimus dose in pediatric patients should be increased gradually for the first 3 weeks after LDLT, whereas adult patients would require dose escalation for the initial 2 weeks. In the covariate analysis, CL/F was found to be related to SCR, TBIL, and ALB. These factors have been demonstrated as significant covariates for tacrolimus clearance in other