Pharmacokinetics of Tacrolimus in Living Donor Liver Transplant and Deceased Donor Liver Transplant Recipients

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Introduction. Hepatic dysfunction is an important determinant of the clearance of tacrolimus; however, the impact of reduced hepatic mass in living donor liver transplant (LDLT) patients on the drug exposure and clearance of tacrolimus is not known.

Aim. The aim of the present study is to compare the dosage, concentration and pharmacokinetics parameters of tacrolimus between LDLT and deceased donor liver transplant (DDLT) recipients.

Patients and Methods. Daily doses used and trough concentrations measured were compared in 12 LDLT and 12 DDLT patients. Multiple blood samples were taken over one dosing interval after oral tacrolimus administration, and pharmacokinetics differences were compared.

Results. The mean tacrolimus dosage in first 14 postoperative days was (0.06 mg/kg/day) for LDLT and (0.09 mg/kg/day) for DDLT ($P=0.0001$). Despite the lower doses used, mean trough concentration was significantly greater in LDLT as compared with DDLT (8.8±2.5 ng/mL vs. 6.79±1.5 ng/mL, respectively, $P=0.013$). On the day of the pharmacokinetic study, minimum Concentration (Cmin), 12-hr postdose concentration (Clast), and average concentration (Cavg) were significantly greater in LDLT as compared with DDLT (LDLT: 6.6±2.4 ng/mL, 7.2±1.8 ng/mL, 8.9±3.0 ng/mL; DDLT: 4.3±1.0 ng/mL, 4.9±1.6 ng/mL, 5.9±1.4 ng/mL, $P=0.02$, 0.04, and 0.02, respectively). Dose normalized AUC was 37.7% greater and clearance, 47.5% lower in LDLT as compared with DDLT.

Conclusion. Although not statistically significant, the dose normalized AUC was 37.7% greater and clearance 47.5% lower in LDLT as compared with DDLT. An initial tacrolimus dose reduction of about 30–40% may be prudent in LDLT compared with DDLT recipients.

Keywords: Tacrolimus, Liver transplantation, Live donor, Deceased donor, Pharmacokinetics.

(*Transplantation* 2008;85: 554–560)

Tacrolimus is a macrolide that was introduced into clinical trials in 1989. It is 10 to 100 times more potent than cyclosporin and provides a reduced rate and severity of acute and chronic rejection after successful liver transplantation (LTx) (*1–6*). Several kinetic studies of tacrolimus have been performed in deceased donor liver transplant (DDLT) patients (*7–9*). It was clear that the oral absorption of tacrolimus is incomplete, unpredictable, and variable, not only between individuals but also within the same individual at different time points after liver transplantation (*10*, *11*). Tacrolimus is primarily cleared in the liver by the hepatic cytochrome P450/3A (CYP3A) enzymes (*12–16*). Hepatic dysfunction has been shown to impair the elimination of tacrolimus, and tacrolimus blood concentrations need to be monitored to minimize significant clinical toxicity (*7*, *8*, *17*, *18*). Drugs that are metabolized through CYP3A enzymes are known to interfere with the metabolism of tacrolimus (*19–28*). Children metabolize the drug faster and need a greater dose based on the body weight (*29–31*).

With the shortage of DDLT, more live donor liver transplants (LDLTs) are being performed in the western world. Although the DDLT recipient receives the complete hepatic allograft, an adult LDLT recipient receives only 55–65% of hepatic mass. One would anticipate a greater drug exposure in LDLT patients compared with DDLT patients, given the same dose of drugs that are metabolized in the liver. Limited information is available on the differences in the pharmacokinetics of drugs in LDLT and DDLT. LDLT patients have been shown to require smaller doses of tacrolimus compared with DDLT (*32–35*). Most of these studies used a nonspecific immunoassay to measure tacrolimus concentrations. The aim of the present study is to compare the pharmacokinetics parameters of tacrolimus along with dosage and concentration differences between LDLT and DDLT patients in a single center using a highly specific and sensitive analytical methodology for measurement of tacrolimus.

PATIENTS AND METHODS

Between January 2005 and November 2005, 12 consenting adult LDLT and 12 adult DDLT patients were prospectively enrolled in an institutional review board-approved protocol to study the pharmacokinetics of tacrolimus after oral administration. Patients with retransplant or multiple transplants were not eligible to participate in this study. Characteristics of all the patients with primary diagnosis are given in Table 1. The mean height, weight, body mass index, and

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Received 23 August 2007. Revision requested 12 September 2007.
Accepted 27 November 2007.

ISSN 0041-1337/08/8504-554
DOI: 10.1097/TP.0b013e3181642c95

TABLE 1. Characteristics of patients LDLT vs. DDLT

	LDLT	DDLT
Male	7	11
Female	5	1
Mean age recipients, years	51.6±6.7	51.5±13.1
Mean age donor, years	38.3±11.6	51.3±13.5
Mean weight, kg	88.1±17.2	86.9±22.6
Mean height, cm	172.1±10.0	174.7±5.3
Body mass Index, kg/m^2	30.6±8.1	29.11±6.9
Mean body surface area, m^2	2.1±0.20	2.06±0.27
Diagnosis		
Hepatitis C	5	5
Ethanol	4	3
Cryptogenic	2	1
Primary biliary cirrhosis	1	0
Auto Immune	0	1
Sclerosing cholangitis	0	1
Biliary Atresia	0	1
Blood Type		
O	8	5
A	3	3
B	1	1
AB	0	3

body surface area were comparable in DDLT and LDLT recipients. However, there was a preponderance of males in the DDLT group, and the mean age of the donor was lower in LDLT compared with DDLT (P=0.019). All the patients received oral tacrolimus, starting at a dose of 0.04±0.05 mg/kg twice a day. The dose of tacrolimus was adjusted as per the clinical conditions, and the target trough levels were normally maintained around 6–10 ng/mL during the first four weeks after transplantation.

All patients received intravenous MMF at a dose of 1 g twice per day (constant two hour infusion) for 2 to 8 days and then converted to oral MMF 1 g twice a day when oral feeds were resumed. All patients also received 500 mg of methyl prednisolone before perfusion of the liver and then a total of 600 mg methyl prednisolone that was tapered over the next 5 days (day 1: 100 mg bid, day 2: 80 mg bid, day 3: 60 mg bid, day 4: 40 mg bid and day 5: 20 mg bid). None of the patients were on any drug, which is known to interfere with tacrolimus metabolism during the study period of first 14 postoperative days.

The daily total dose of tacrolimus, cumulative dose of tacrolimus for first 14 postoperative days and morning trough concentration of the drug were compared. Values are presented as mean and standard deviation. Serial blood samples were drawn for the study participants at 0 (predose), 1, 2, 3, 4, 5, 6, 8, 10, and 12 hr from the fourth to tenth postoperative day after the patient was on a stable dose of tacrolimus for at least 2 days. Blood samples were drawn in BD Vacutainer® tubes (Franklin Lakes, NJ) spray-coated with K_2EDTA. The whole blood concentration of tacrolimus was measured using a high-performance liquid chromatographic mass spectrometry method (HPLC/MS/MS) developed by Volosov and Soldin (*36*). Whole blood samples were deproteinated with acetonitrile containing ritonavir as internal standard. Supernatants were injected onto a HPLC (Agilent Technologies, Palo Alto, CA) equipped with a Supelcosil LC-18-DB column (Supelco, Bellefonte, PA.). Tacrolimus was quantitated with a API-2000 tandem mass spectrometer (Applied Biosystems, Foster City, CA) using atmospheric pressure chemical ionization (APCI). The assay precision is 9.6% at a drug concentration of 8 ng/mL. Daily trough levels of tacrolimus were also measured. Liver function, renal function, and daily changes in tacrolimus doses were prospectively cataloged. Various pharmacokinetics parameters were calculated using non-compartmental analysis with WinNolin software (version 4.1, Pharsight Corporation, Mountainview, CA).

The parameters observed included time to reach maximum concentration (Tmax), minimum concentration (Cmin), maximum concentration (Cmax), 12-hr postdose concentration (Clast), area under the concentration vs. time curve (AUC), apparent oral clearance (CLss_F), and average steady-state concentration (Cavg). Differences in mean parameters were compared with t test using SPSS software Windows based version 14.0 (SPSS, Chicago IL). $P<0.05$ was considered statistically significant. Unfortunately, the weight of the liver was recorded only in 6 DDLT and 10 LDLT patients, although it was a part of the protocol. The mean graft-weight ratio in DDLT was 1.69±0.73 (n=6) and, in LDLT, it was 1.06±0.29 (n=10). Thus the mean graft-weight ratio in DDLT was about 1.6 times higher compared with LDLT.

RESULTS

Daily Tacrolimus Dosage and Trough Level Comparison

One DDLT patient had hepatic artery thrombosis, and tacrolimus was discontinued after the first postoperative day until he was retransplanted a week later. Hence, 11 DDLT patients were compared with 12 LDLT patients for daily dosing of tacrolimus and morning trough concentrations. LDLT and DDLT were commenced on the same dose of tacrolimus postoperatively. However, subsequent daily dose reduction and discontinuation in LDLT were much more frequent compared to DDLT.

The mean daily tacrolimus dose for first 2 weeks after liver transplant was significantly lower in LDLT compared with DDLT (5.4±0.9 mg/day; 0.06 mg/kg/day vs. 7.8±1.7 mg/day; 0.09 mg/kg/day, respectively, P=0.0001; Fig. 1a). Also, the mean cumulative dose for the first 14 days was significantly lower in LDLT compared with DDLT (69.9±36.7 mg; 0.78 mg/kg in LDLT vs. 118.5±60.2 mg; 1.30 mg/kg in DDLT; P=0.028; Fig. 1b). Despite the significantly lower mean daily dosages in LDLT compared with DDLT, the mean morning trough concentration of tacrolimus for the first 2 weeks after liver transplantation was significantly greater in LDLT compared with DDLT (8.8±2.5 ng/mL in LDLT vs. 6.7±1.5 ng/mL in DDLT; P=0.013; Fig. 2a). At the same time, mean total bilirubin values during the first 14 postoperative days were significantly lower in LDLT (2.6±0.7 mg/dL) compared with DDLT (4.2±0.7 mg/ dL, P=0.001, Fig. 2b).

Pharmacokinetics

Out of the 24 patients enrolled in the study, in 5 cases the pharmacokinetics study could not be performed (4 LDLT,

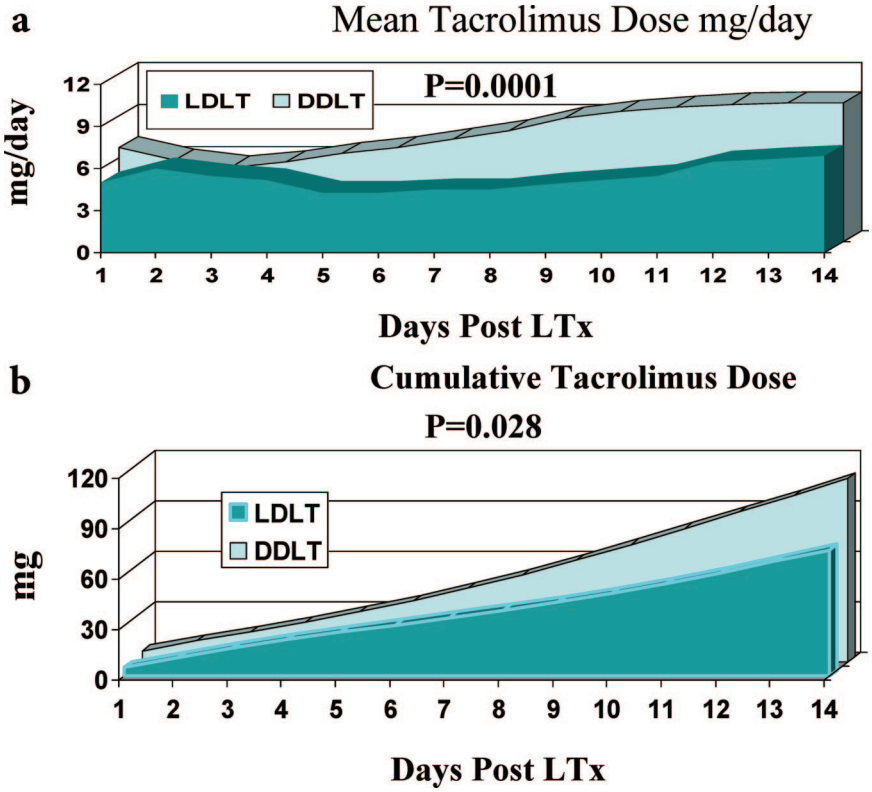

FIGURE 1. (a) Mean daily dose of tacrolimus for LDLT and DDLT. (b) Cumulative tacrolimus dose over time in LDLT and DDLT.

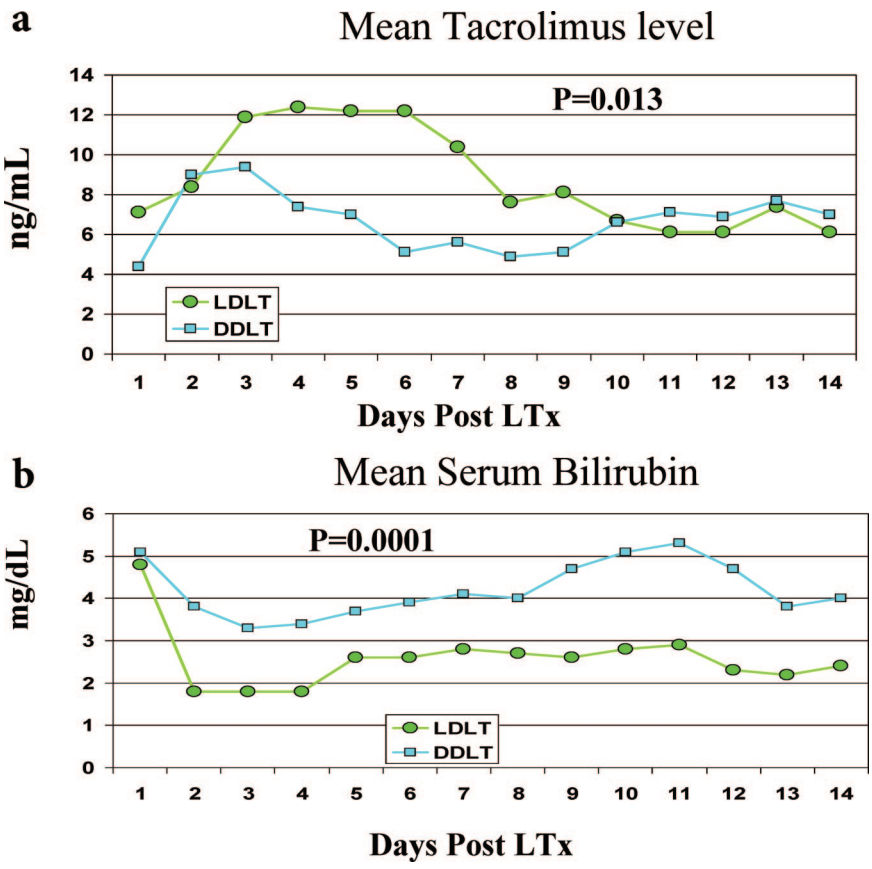

FIGURE 2. (a) Mean daily trough tacrolimus concentration in nanograms per milliliter for LDLT and DDLT. (b) Mean total daily serum bilirubin in nanograms per milliliter in LDLT and DDLT.

TABLE 2. Mean Biochemical parameters on the kinetics day

	WBC, 10^3/mL	Hct, %	Plat, 10^3/mL	BUN, mg/dL	Creat, mg/dL	Alb, g/L	T Bili, mg/dL	AST, U/L	ALT, U/L	ALK, U/L	GGT, U/L
LDLT											
Mean	14.1	25.3	122.3	54.6	1.8	2.3	2.9	67.1	183	121.6	269.3
SD	7.5	4.5	49.1	42.7	1.8	0.1	2	58.1	135.6	54.3	148.7
DDLT											
Mean	7.4	29.9	89.6	35.5	1.5	2.6	2.5	60.6	174.3	126.9	298.2
SD	3.8	5.7	73.6	23.1	1.3	0.4	1.7	57.9	94.9	40.4	105.2

WBC, white blood cell count; Hct, hematocrit; Plat, platelet count; BUN, blood urea nitrogen; Creat, creatinine; Alb, albumin; T Bili, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALK, alkaline phosphatase; GGT, gamma glutamyl transpeptidase.

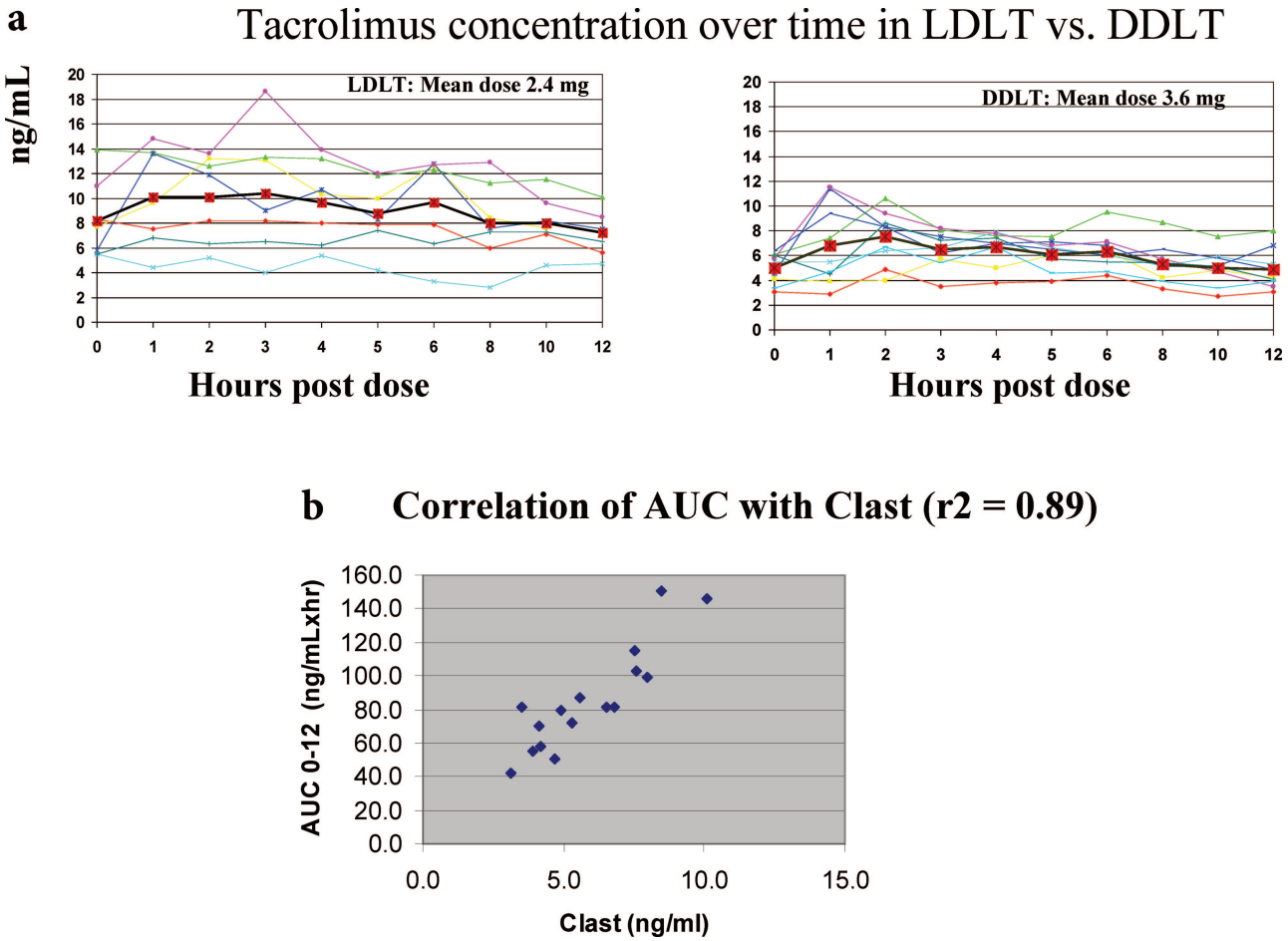

FIGURE 3. (a) Tacrolimus concentration over time in DDLT and LDLT for individual patient. Solid line represents mean value. (b) Correlation between trough blood concentration and AUC (n=16).

1 DDLT), because they were not on a steady dose of the drug (n=5) within the study period. One patient had a lack of venous access (LDLT#12), one patient's samples were contaminated (DDLT#4), and one patient had an undetectable concentration of tacrolimus on most of the samples and could not be evaluated (DDLT#3). Hence, complete pharmacokinetics data could be calculated in 7 LDLT and 9 DDLT recipients. The pharmacokinetics studies were conducted on mean postoperative day 7.5±1.9 (median 7.5, range 4 to 10) for LDLT and 7±1.5 days (median 7, range 5 to 9) for DDLT. The mean tacrolimus dose on the day of kinetics study in LDLT was 2.4±0.98 mg and in DDLT, it was 3.6±3.1 mg.

The biochemical and hematological profile of the study patients on the day of pharmacokinetics study is shown in Table 2. Liver enzymes, bilirubin, albumin, and hematocrit were comparable between the 2 groups. Tacrolimus blood concentrations over time for each patient in both groups are shown in Figure 3a (left-LDLT, right-DDLT; solid line represents the mean value in each group). In general, even at lower

TABLE 3. Kinetics parameters LDLT vs. DDLT

Parameters	Tmax, hr	Cmax, ng/mL	Cmin, ng/mL	Clast, ng/mL	Cavg, ng/mL	AUC 0–12, ng/mLxhr	AUC 0–12/ dose, ng/mLxhr/mg	CLss_F, mL/hr
LDLT								
L1	0.0	8.3	5.6	5.6	7.3	87.4	43.7	22883
L2	2.0	13.2	7.6	7.6	9.7	103.4	25.9	38703
L3	0.0	13.9	10.1	10.1	12.1	145.5	48.5	20619
L7	0.0	5.5	2.8	4.7	4.2	50.4	25.2	39682
L10	1.0	13.6	5.8	7.5	9.6	114.7	38.3	26155
L11	3.0	18.6	8.5	8.5	12.6	151.0	75.5	13249
L12	5.0	7.4	5.5	6.5	6.8	81.1	81.1	12330
Mean		11.5	6.6	7.2	8.9	104.8	48.3	24803
SD		4.6	2.4	1.8	3.0	35.9	22.3	11005
DDLT								
D1	2.0	4.9	2.7	3.1	3.5	42.3	42.3	23669
D2	6.0	6.4	3.9	4.2	4.9	58.5	19.5	51326
D5	2.0	10.6	6.1	8.0	8.2	98.9	14.1	70779
D6	4.0	7.9	5.2	5.3	6.0	72.3	14.5	69204
D7	1.0	11.4	4.6	6.8	6.8	81.2	40.6	24630
D8	1.0	11.5	3.5	3.5	6.8	81.6	8.2	122624
D10	2.0	8.6	4.1	4.1	5.8	69.8	69.8	14337
D11	1.0	9.4	4.9	4.9	6.6	79.1	79.1	12642
D12	2.0	6.7	3.4	3.9	4.6	55.4	27.7	36134
Mean	2.3	8.6	4.3	4.9	5.9	71.0	35.1	47261
SD	1.7	2.3	1.0	1.6	1.4	16.9	25.3	35717
Mean difference LDLT vs. DDLT (%)		+33.7	+53.7	+48.2	+50.6	+47.6	+37.7	−47.5
P value		0.12	0.02	0.04	0.02	0.03	0.29	0.11

Tmax, time to reach maximum concentration; Cmax, maximum concentration; Clast, 12 h postdose concentration; AUC 0–12, area under curve up to last data point; Cmin, minimum concentration; Cavg, steady-state average concentration; CLss_F, apparent oral clearance at steady state.

doses, blood concentrations in LDLT patients tended to be higher than those observed in DDLT patients.

There was a good correlation (r^2=0.89) between the trough tacrolimus blood concentrations and the corresponding AUC when all the patients were evaluated (Fig. 3b, n=16), which suggests that patients were in a steady state at the time of kinetics study.

Pharmacokinetics parameters for each LDLT and DDLT patient are shown in Table 3. The mean maximum concentration (Cmax) was 33.7% greater in LDLT compared with DDLT (LDLT: 11.5±4.6 ng/mL, DDLT: 8.6±2.3 ng/mL, *P*=0.121) and the mean minimum concentration (Cmin) was 53.7% greater in LDLT compared with DDLT (LDLT: 6.6±2.4 ng/mL, DDLT: 4.3±1.0 ng/mL, *P*=0.021), which was significant. Mean 12-hr postdose concentration (Clast) was 48.2% greater in LDLT compared with DDLT (LDLT: 7.2±1.8 ng/mL vs. DDLT: 4.9±1.6 ng/mL, *P*=0.04). The mean average concentration (Cavg) was significantly greater (50.6%) in LDLT compared with DDLT (LDLT: 8.9±3.0 ng/mL, DDLT: 5.9±1.4 ng/mL, *P*=0.021). The mean area under the concentration curve for 12 hr (AUC 0–12) was 47.6% greater in LDLT compared with DDLT (LDLT: 104.8±35.9 ng/mLxhr, DDLT: 71.0±16.9 ng/mLxhr, *P*=0.02), which was significant. However, the mean dose normalized AUC 0–12/dose (ng/mLxhr), although 37.7% greater in LDLT compared with DDLT (LDLT: 48.3±22.3 ng/mLxhr, DDLT: 35.1±25.3 ng/mLxhr), it did not reach statistical significance (*P*=0.28). Also, the apparent clearance of the drug was 47.5% lower in LDLT compared with DDLT (LDLT: 24803±11005 mL/hr, DDLT: 47260±35717 mL/hr) but did not reach statistical significance.

DISCUSSION

Although increasing numbers of LDLT are being performed in United States and elsewhere, the impact of reduced size/mass of the hepatic allograft and the process of hepatic regeneration, on the pharmacokinetics parameters of drugs used in the LDLT patients have not been thoroughly evaluated. This information is especially important for proper dosing of immunosuppressive drugs such as cyclosporin and tacrolimus. Given that tacrolimus has a narrow therapeutic index, it is important to monitor the blood concentrations of this drug in transplant patients. The implications and side effects from overdosing of the drug in a large population during the early phase of the drug development have been reported earlier (*37*). Similar errors may be avoided in LDLT patients and these patients could be protected from nephrotoxicity, neurotoxicity, hyperglycemia and risk of infection by appropriate adjustment of the starting dosage of tacrolimus in LDLT recipients.

Liver is the primary site for the metabolism of tacrolimus and ciclosporin, although some of the drug is also metabolized in the gut (*38*). Hepatic dysfunction significantly impairs the elimination of tacrolimus (*7*, *8*, *17*, *18*). The functional status of the liver, as modified by the presence of other co-administered drugs, also is known to alter the trough levels and kinetics profiles of tacrolimus (*19–27*, *39*). However, an evaluation of the impact of smaller volume of liver (reduced hepatic mass) and its impact on AUC of the drug and various pharmacokinetics parameters in LDLT patients in comparison to DDLT patients have not been previously reported.

Only limited studies in small numbers of patients have characterized the fate of tacrolimus in LDLT. These reports are primarily limited to the analysis of the trough blood concentrations of tacrolimus (*32–35*). All the previously published pharmacokinetics studies on tacrolimus in LDLT have used an enzyme immunoassay method, where the metabolites of tacrolimus also cross react (*40*). The amount of metabolites of tacrolimus varies with hepatic dysfunction when assay detects both metabolites of tacrolimus and tacrolimus. Both the values are higher as compared to HPLC-MS, where only parent compound of tacrolimus is used. This may have clinical advantage particularly when there is hepatic dysfunction (*41*, *42*). Greater tacrolimus blood concentrations have been reported in LDLT recipients compared with DDLT recipients (*43*, *44*). Charco et al. compared AUC in LDLT and DDLT; however, the steroid dose was not uniform in all patients and the method of calculation of AUC was not clear (*45*). The clearance of tacrolimus on day 30 after LDLT has been reported to be lower than what is reported in healthy subjects (*46*). Previous studies have suggested a relationship between tacrolimus clearance with body weight and postoperative days (*47*). There is a correlation between the graft weight to recipient liver volume ratio and the dose needed to maintain tacrolimus blood levels (*48*).

Although the aforementioned reports suggest that LDLT recipients need smaller dosage based on trough concentration, in this study LDLT and DDLT have been compared prospectively during the first 14 days postLTx, with pharmacokinetics studies in between the dosing intervals. In the current study, despite significantly lower dose of tacrolimus in LDLT, the mean trough concentrations of tacrolimus for the first 14 postoperative days were significantly greater compared with DDLT. Our study has shown that the dose-normalized AUC while tended to be higher (37.7%), it did not reach statistical significance. During the study period trough concentration of tacrolimus and bioavailability of oral and intravenous tacrolimus was found to be higher with hepatic dysfunction in DDLT compared with recipient with good liver function. This was thought to be caused by the impairment of metabolic pathway of CYP4503A system. In the present series, mean total bibirubin in DDLT was significantly greater compared with LDLT. On the basis of previous reported experience, LDLT should have had lower trough level and lower AUC compared with DDLT; hence, we feel that pharmacokinetic profile of tacrolimus seems more dependent on liver mass than function.

Our study is limited in two respects. Because of the frequent reduction and discontinuation of tacrolimus dosage in LDLT patients, kinetics studies could not be performed for lack of steady state concentrations in the intended study population. Also, a large variation in the apparent clearance of tacrolimus was observed in this patient population. Assuming a 50% increase in AUC/1 mg dose of tacrolimus to be clinically significant, based on the observed variation in AUC, 34 subjects are needed in each group. This observation points to the importance of conducting such collaborative studies in multiple centers. Because intravenous tacrolimus is not routinely used in liver transplant patients, it was not possible to compare the systemic clearance of tacrolimus in LDLT and DDLT patients.

It is interesting to note that the mean total bilirubin concentrations in DDLT were significantly greater compared with LDLT. Previous observations in patients with hepatic dysfunction suggest a need for the use of lower doses of tacrolimus in patients with greater bilirubin (*7*, *8*, *17*, *18*). The lower functional capacity of the liver in the DDLT recipient might have blunted the possible differences in clearance between the LDLT and DDLT patients. The observed tendency for higher trough concentrations supports the use of lower daily dosing of tacrolimus in LDLT compared to DDLT. Because the AUC/1 mg dose of tacrolimus is approximately 38% greater in LDLT patients, the dose of tacrolimus can be decreased by 30–40% and further dose changes must be guided by therapeutic monitoring of tacrolimus.

CONCLUSION

We have shown that, despite better hepatic function (as determined by lower bilirubin levels) in the immediate postoperative period, the mean whole blood concentration, the mean 12 hr postdose concentration, and AUC were significantly greater in LDLT patients compared with DDLT patients. Although dose normalized AUC was 37.7% greater and apparent clearance 47.5% lower in LDLT compared to DDLT, it did not reach statistical significance. In LDLT recipients during the early postoperative period, tacrolimus toxicity and over-immunosuppression may be minimized by preemptively starting these patients at a dose that is approximately 30–40% lower compared with DDLT and further adjusting the dose based on tacrolimus trough concentration.

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