

Population Pharmacokinetic Estimation of Tacrolimus Apparent Clearance in Adult Liver Transplant Recipients

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Abstract: The goal was to study the factors affecting tacrolimus apparent clearance (CL/F) in adult liver transplant recipients. Tacrolimus dose and concentration data (n = 694) were obtained from 67 liver transplant recipients (22 female and 45 male), and the data were analyzed using a nonlinear mixed-effect modeling (NONMEM) method. A 1-compartment pharmacokinetic model with first-order elimination, an absorption rate constant fixed at 4.5 hours⁻¹, and first-order conditional estimation was used to describe tacrolimus disposition. The predictive performance of the final model was evaluated using data splitting and assessing bias and precision of the estimates. The population estimate of tacrolimus CL/F and apparent volume of distribution (V/F) were found to be 21.3 L/h (95% confidence interval, CI, 18.0–24.6 L/h) and 316.1 L (95% CI 133–495 L), respectively. Neither patient's age, weight, gender, nor markers of liver function influenced tacrolimus CL/F. The final model was $TVCL = 21.3 + 9.8 \times (1 - HEM) + 3.4 \times (1 - ALB) - 2.1 \times (1 - DIL) - 7.4 \times (1 - FLU)$, where TVCL, typical estimate of apparent clearance, HEM = 0 if hematocrit <35%, otherwise 1; ALB = 0 if albumin <3.5 g/dL, otherwise 1; DIL = 0 if diltiazem is coadministered, otherwise 1; FLU = 0 if fluconazole is coadministered, otherwise 1. This study identified the factors that significantly affect tacrolimus disposition in adult liver transplant recipients during the early post-transplantation period. This information will be helpful to clinicians for dose individualization of tacrolimus in liver transplant recipients with different clinical conditions including anemia or hypoalbuminemia or in those patients receiving diltiazem or fluconazole.

Key Words: albumin, hematocrit, liver transplant, population pharmacokinetics, tacrolimus

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Tacrolimus, a macrolide lactone, has been used as a first-line immunosuppressant in liver and kidney transplant recipients. In spite of its success in ensuring graft survival, therapeutic use of tacrolimus is complicated by its narrow therapeutic index, wide intra- and interpatient variability^{1–3} and the risk of drug interactions with concurrently used medications. To reduce toxicity and increase graft survival in transplant recipients, trough whole blood concentration of tacrolimus is routinely monitored as a guide for dosage adjustment.^{3–7} However, there is a poor correlation between tacrolimus dose and trough concentrations, supporting the need for additional information on the factors influencing the pharmacokinetic characteristics of tacrolimus.

A number of studies have been performed to characterize the pharmacokinetics of tacrolimus in kidney and liver transplant recipients.^{2,8,9} After ingestion, tacrolimus is rapidly absorbed with an absorption rate constant of 4.5 ± 3.0 hours⁻¹, giving a peak concentration within 0.5 to 1 hour.² In whole blood tacrolimus is mainly associated with erythrocytes (80%–95%), and in plasma it predominantly binds to soluble proteins such as albumin and α₁-acid glycoprotein, and to a lesser extent to lipoproteins.^{10,11} It is extensively metabolized in the intestinal mucosa and liver mainly by enzymes belonging to CYP3A4 system.^{12,13} Tacrolimus has an apparent clearance of around 20–30 L/h, and several studies suggest that tacrolimus clearance is decreased in patients with hepatic dysfunction.^{2,3,14,15}

Although conventional pharmacokinetic studies have estimated tacrolimus pharmacokinetic parameters including clearance, volume of distribution, and absorption rate constant, these studies by design were unable to describe the factors that affect the variability in tacrolimus pharmacokinetics. A number of researchers have used a population approach to study tacrolimus pharmacokinetics in both adult and pediatric transplant recipients.^{3,5,6} However, because of lack of availability of hematological, biochemical, and other clinical data in these analyses, a detailed investigation of the factors affecting the variability in tacrolimus pharmacokinetics is still needed.

The primary aim of this study was to develop a population pharmacokinetic model to estimate the value of tacrolimus apparent clearance and to assess the intra- and interpatient variability in this parameter in a cohort of adult liver transplant recipients. The effects of concomitant medications in addition to various demographic, hematological, and biochemical parameters on tacrolimus disposition was also investigated.

METHOD

Patients and Data Collection

Data were collected retrospectively for 67 adult patients who underwent liver transplantation from 1997 to 2001 at the Royal Prince Alfred Hospital, Sydney, NSW, Australia. The data collection for the present study was approved by the University of Sydney and Central Sydney Ethics Committees. Permission for data transfer and analysis was also obtained from the University of Sydney. The Institutional Review Board at the University of Rhode Island, USA, has also approved the population pharmacokinetic analysis of the data.

Data and relevant patient information were obtained from the patient medical record files (demographic data, medication history, and clinical events) and inpatient clinical records (dosage regimen and concentrations of tacrolimus) using a standardized data collection form. The timings of tacrolimus dose intake and blood sampling were noted by the nurse in charge of patient management in the inpatient record. The dataset included tacrolimus dose, trough blood concentrations, dose history, and time since the last dose. Patient demographic characteristics including age, weight, height, gender, and ethnicity and information on concomitantly administered medications were collected. In addition, the data on biochemical and hematological indices including alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyl transferase (GGT), bilirubin, albumin, total protein, and total cholesterol concentrations and hematocrit were also collected. Data were collected only for the duration of inpatient hospitalization.

Drug Administration and Therapeutic Drug Monitoring

Oral tacrolimus (PrografTM capsules, 1 mg and 5 mg, Janssen-Cilag, Sydney, NSW, Australia) therapy was initiated at a dose between 0.1 and 0.15 mg/kg/d (in 2 divided doses), usually within 48 hours (13.4 ± 7.6 hours, range 8.0–23.0 hours) after transplant surgery when an adequate level of renal function was reestablished (creatinine clearance approximately 60 mL/min). Tacrolimus dose was then adjusted to maintain blood trough concentrations within the target range of 10 to 15 ng/mL or based on the clinical status of the patient. Unless otherwise specified, the standard practice at the Liver Transplant Unit at the time of the study was to administer tacrolimus doses at 8 AM and 8 PM. For monitoring tacrolimus blood concentration, blood samples were collected between 7 AM and 8 AM on Monday, Wednesday, and Friday, unless additional monitoring was advised by the transplant physician. Blood samples were collected in Beckton Dickinson Vacutainer[®] tubes containing EDTA and analyzed by the Department of Pathology, Royal Prince Alfred Hospital, using the Tacrolimus-2 microparticulate enzyme immunoassay (MEIA[®]) method run on the IMx[®] analyzer (Abbott Laboratories, Abbott Park, IL). The coefficient of variation of the assay (%CV) was 8.4% and 14.5% at concentrations of 1.9 and 29.5 ng/mL, respectively, with a working concentration range between 1.5 ng/mL and 30 ng/mL. Biochemical and hematological indices were monitored every day during the patients' hospital stay.

More than 4000 dose and 710 tacrolimus concentration observations were extracted from the Transplant Unit's therapeutic drug-monitoring database. However, after an audit of the available data only 694 observations were included in the population analysis. Sixteen concentrations were excluded from the analysis because they were outside the working range of the assay. Most of the concentrations were collected toward the end of the dosing interval (average time after dose 9.8 ± 1.5 hours, Table 1). For patients receiving tacrolimus as the primary immunosuppressant, data were collected over an average of 40.2 ± 17.3 posttransplantation days (range 14 to 94 days). Twelve patients received tacrolimus as rescue therapy, out of which 9 patients had ongoing rejection and 3 patients experienced nephrotoxicity associated with cyclosporine therapy. Data were obtained for these patients for 23.3 ± 15.4 days after the conversion of the immunosuppressant therapy to tacrolimus.

Immunosuppressants and Other Maintenance Therapy

All patients were receiving tacrolimus twice a day as part of their dual, triple, or quadruple immunosuppressant regimen comprising tacrolimus and prednisolone with or without other agents including azathioprine ($n = 33$), sirolimus ($n = 2$), mycophenolate mofetil ($n = 8$), and OKT3 (anti-CD3 monoclonal antibody) ($n = 4$). More than 50% of the patients were receiving omeprazole (20 mg/d or 40 mg/d), and 1 patient was receiving pantoprazole. Approximately 46%, 15%, and 10% of the patients were receiving fluconazole (100 to 400 mg/d), diltiazem, and phenytoin, respectively. Patients were also receiving a number of other medications including antihypertensive agents (40.3%), oral hypoglycemic agents (22%), insulin (19%), antibacterial (27%) and antiviral (32%) agents, depending on their clinical need.

TABLE 1. Demographic and Biochemical Data of Study Population ($n = 67$)

Variables	Value	Range
Gender (% male)	67.2	—
Age (yr)	$46.2 \pm 12.7\#$	14–67.5
Weight (kg)	$77.8 \pm 17.4\#$	50.0–137.3
Time posttransplant (d)	$40.2 \pm 17.3\#$	14–94
Observations per patient	$10.3 \pm 5.8\#$	1–35
Dose of tacrolimus (mg/d)	$7.6 \pm 4.8\#$	1–24
Tacrolimus blood concentration (ng/mL)	$11.1 \pm 5.6\#$	1.5–29.5
Sample time (time after dose, h)	9.8 ± 1.5	6–13
Hematocrit (%)	41*	25–46
Albumin (g/dL)	4.3*	2.3–5.2
Total cholesterol (mmol/L)	4.8*	3.6–6.6
ALT (U/L)	149*	14–5450
AST (U/L)	51*	8–4830
GGT (U/L)	298*	13–6382
Bilirubin (μ mol/L)	39*	5–614
Creatinine (μ mol/L)	74.5*	18–803

#Mean \pm SD, *median.

Population Pharmacokinetic Modeling

Population pharmacokinetic modeling was performed using NONMEM (version 5, level 1.1, GloboMax LLC, Hanover, MD) with double-precision and first-order conditional estimation (FOCE) technique. PDx-POP (version 1.1, GloboMax LLC, Hanover, MD) was coupled with NONMEM as an interface. FORTRAN 77 (Hewlett Packard, USA) was used for data compilation, and the whole system was run on an IBM-compatible personal computer loaded with Windows XP as an operating system. Because only tacrolimus blood concentrations at the end of the dosing interval were available, a 1-compartment pharmacokinetic model (NONMEM subroutine ADVAN 2 and TRANS2) with first-order absorption and elimination was used to describe the concentration–time data (Equation 1):

$$C_p = \frac{F \text{Dose } k_a}{V(k_a - k_{el})} [e^{-k_{el}t} - e^{-k_a t}] \quad (1)$$

where C_p is blood or plasma concentration, F is oral bioavailability, Dose is the last dose of tacrolimus, k_a is absorption rate constant in hours^{-1} , V is volume of distribution, k_{el} is elimination rate constant, and t is the time of sample collection with respect to the time of the dose.

The model has also been employed by several other research groups in the population pharmacokinetic analysis of tacrolimus.^{3,6} Empiric Bayes estimates of the model parameters for each patient were obtained using POSTHOC option in NONMEM. Because no data from the absorption phase were available, absorption rate constant (k_a) was fixed. The effect of fixing the k_a using a stepwise increase in k_a value from 0.5 to 8.0 hours^{-1} was evaluated. Preliminary analyses indicated that k_a fixed at 4.5 hours^{-1} yielded the minimum value of the NONMEM derived objective function and the smallest coefficient of variance for the parameter (CL/F, V/F) estimates. Because similar k_a values for tacrolimus had also been reported in the literature,^{2,16} for subsequent analyses k_a was fixed at 4.5 hours^{-1} . Initial estimates for other parameters were obtained from the literature.³⁻⁶

Estimation of apparent clearance (CL/F) of tacrolimus using the population pharmacokinetic approach was conducted in 3 steps: base model development and exploratory data analysis, covariate model development followed by selection, and evaluation of the final full-population pharmacokinetic model. The study dataset was randomly split (in PDx-POP) using a 70:30 ratio in the index ($n = 47$, 398 observations) and validation ($n = 20$, 236 observations) datasets used for model development and evaluation, respectively. There was no statistically significant difference ($P > 0.05$) in demographic characteristics (age 44.3 ± 3.6 years vs. 48.2 ± 2.9 years, weight 81.3 ± 12.1 kg vs. 73.1 ± 8.6 kg, gender 68% male vs. 65% male, posttransplantation days 37.2 ± 9.8 days vs. 43.2 ± 7.2 days) of the patients in the index and validation groups.

Base Model Development and Exploratory Data Analysis

In the first step, the base model was developed without including patient covariates using the index dataset. The

minimum objective function value (OFV) obtained in this step was used as a standard for assessing the impact of the inclusion and exclusion of different covariates in the subsequent models.

Individual Bayesian estimates of apparent clearance (CL_j) obtained using this model were further evaluated using graphic and statistical analyses to explore the correlation between apparent clearance and various demographic, concomitant medications, biochemical, and hematological parameters and to identify possible covariates for subsequent analyses.

Different models were evaluated to describe interpatient variability in apparent clearance and residual error (proportional and additive error models). Based on the OFV and the distribution of residuals in the diagnostic plots of the base model, a proportional model for interpatient variability (Equation 2) and an additive model for error distribution (Equation 3) were selected for these analyses, such that:

$$CL_j = TVCL \times \exp(\eta_{CL}) \quad (2)$$

$$C_{ij} = C_{ij\text{PRED}} + \varepsilon_{ij} \quad (3)$$

where CL_j , the estimate of apparent clearance of tacrolimus in j th individual, TVCL, typical value of apparent clearance of tacrolimus (also known as the population estimate), η_{CL} , difference between the true CL_j for the j th patient, and the typical value for the population (TVCL) (also termed as the intersubject variability). C_{ij} is the observed concentration, $C_{ij\text{PRED}}$ the predicted concentration, and ε_{ij} the difference between C_{ij} and $C_{ij\text{PRED}}$. Both η_{CL} and ε_{ij} are randomly distributed with a mean zero and variance of ω^2 and σ^2 , respectively.

Development of a Covariate Model

In the second step, each of the covariates of interest was introduced individually into the base model to assess the relative impact on estimates of apparent clearance. A decrease of OFV (from base model to covariate model) by 6.6 was considered to be statistically significant (at $P < 0.01$ level), and this difference in OFV (between 2 models) was assumed to be asymptotically χ^2 distributed. In the next step statistically significant covariates were included in the base model in a forward selection manner to develop intermediate and full models. A total of 19 covariates were tested in the present study. The continuous covariates evaluated in this study are age (years), weight (kg), ALT (U/L), AST (U/L), GGT (U/L), bilirubin ($\mu\text{mol/L}$), hematocrit (%), albumin (g/dL), total protein (g/dL), total cholesterol (mmol/L), and posttransplantation days. These were evaluated using 4 different approaches including:

$$TVCL = \theta_{CL} + \theta_{cov} * \text{Covariate}$$

$$TVCL = \theta_{CL} + \theta_{cov} * (\text{Covariate} - \text{Median})$$

$$TVCL = \theta_{CL} \times (\text{Covariate})^{\theta_{cov}}$$

$$TVCL = \theta_{CL} \times \text{Covariate}$$

where θ_{CL} is the individual estimate of tacrolimus CL/F, θ_{cov} the factor contributed by the covariate, and TVCL typical value of apparent clearance of tacrolimus (also known as the population estimate). Categorical/discrete covariates including

gender, ethnicity, concurrent medications (fluconazole, phenytoin, diltiazem, omeprazole), diabetic/nondiabetic, and occurrence of rejection were also examined. When other medications (hypoglycemic agent, antibacterial, antiviral agents, etc) were subclassified based on the possible type of interaction with tacrolimus, individual groups did not have sufficient observations (<10%) to be tested for their possible impact on tacrolimus CL/F. For interacting medications, if patients received any of them, they were coded as “0,” and those who did not receive them were coded as “1.” A number of continuous covariates including ALT, AST, GGT, bilirubin, hematocrit, albumin, total protein, total cholesterol, and posttransplantation days were also analyzed after transformation into categorical variables. The cutoff range for data transformation was selected based on clinically accepted criteria. For example, in the case of hematocrit, levels less than 35% were assigned “0,” whereas remaining values were designated as “1.” Furthermore, because most concentration observations were in the later phase of the dosing interval, the effect of covariates on apparent volume of distribution was not modeled.

Selection of Final Full Model

In the final step, covariates were eliminated from the full model developed in the previous step following the backward elimination technique and assessment of significance (at $P < 0.01$). While selecting the final full model, the improvement in fit from addition of a parameter to the model was also assessed by the precision of the parameter estimate (standard error of the mean and 95% confidence interval) and by the reduction in interpatient and residual variability. Scatter plots of weighted residual (WRES) versus tacrolimus predicted concentrations (PRED) were also drawn to check the goodness of fit in each model.

Model Evaluation

Once finalized, the final population pharmacokinetic model, which included influential covariates (with fixed model parameter values), was quantitatively evaluated (bias and precision) using data from the validation dataset (20 patients, 236 observations). Finally, population and posterior Bayesian estimates of apparent clearance of tacrolimus were also estimated using complete ($n = 67$ patients, 694 observations) and validation datasets (without fixing model parameter values). Estimated population parameter values obtained using index, validation, and whole datasets were compared to assess possible impact of data splitting on population estimates of the parameters. Predictive performance (based on bias and precision)¹⁷ of the final model using different datasets was also statistically compared to assess the robustness of the model.

Statistical Analysis

Unless stated otherwise, in the current study all statistical comparisons were done using t test or ANOVA (followed by post hoc analysis) at 0.05 significance level by the use of SPSS version 11.5 software (SPSS Inc., Chicago, IL).

RESULTS

Patient Demographic and Clinical Data

Demographic characteristics of the patient population are presented in Table 1. More than 40% of the patients ($n = 30$) required transplantation because of liver cirrhosis caused by hepatitis B or C infection. Alcoholic cirrhosis was the other major cause ($n = 12$) leading to liver damage and subsequent transplant. Other causes of transplant in these patients included hepatoma, autoimmune liver disease, cryptogenic liver cirrhosis, and biliary atresia. Twenty-four patients were hypertensive, and 17 patients were diabetic before the transplant. The majority of the patients were white (82%), approximately 10% were Asian, and 8% were from African origin.

Tacrolimus Dose and Trough Blood Concentration

The average daily dose and trough blood concentration of tacrolimus varied widely over the posttransplantation period with an average of 7.6 ± 4.8 mg/d (in 2 divided doses) and 11.1 ± 5.6 ng/mL, respectively (Table 1). No correlation was observed between tacrolimus dose and trough blood concentration ($r^2 = 0.014$) (Fig. 1).

The average daily dose of tacrolimus ranged from 1 to 24 mg/d over the posttransplantation period. Although there was high intra- and interindividual variability in tacrolimus trough blood concentration in the study cohort, no significant change in tacrolimus trough concentration was detected over the study period ($P = 0.44$). However, there was a significant decrease (by approximately 30%, 95% CI 21.9%–39.0%, $n = 45$) in the ratio of tacrolimus daily-dose (mg/d)-to-trough-concentration (ng/mL) detected from posttransplantation day 30 onwards (1.0 ± 0.5 vs. 0.7 ± 0.3 , $P = 0.004$, Fig. 2).

Base Pharmacokinetic Model

The distribution of observed and base model (1-compartment pharmacokinetic model with exponential

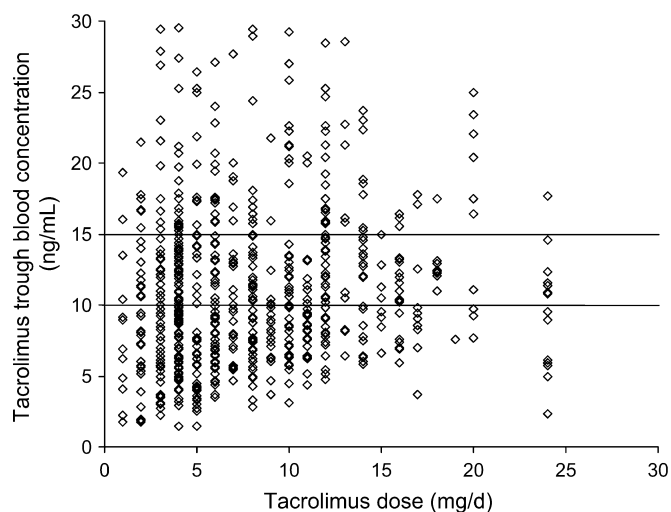


FIGURE 1. Tacrolimus daily dose versus trough blood concentration over the posttransplantation period for 67 patients. Solid lines represent target trough blood concentration of tacrolimus.

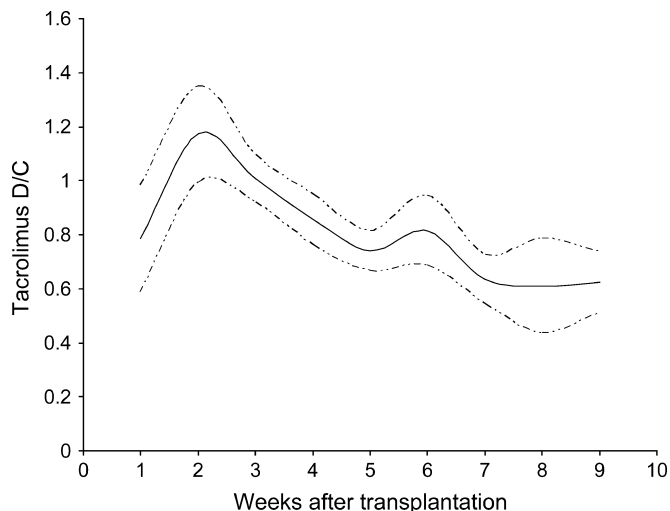


FIGURE 2. Change in the ratio (D/C) of tacrolimus daily dose (mg/d) to trough blood concentration (ng/mL) over the posttransplantation period for 67 patients. Solid line represents mean value, and the dashed lines represent upper and lower limits of the 95% confidence interval.

distribution of intersubject variability and additive residual error distribution) predicted tacrolimus trough blood concentrations (for the complete dataset) is presented in Figure 3. The population estimates of tacrolimus CL/F and V/F using the base model are 23.7 L/h and 389.2 L, respectively. Interpatient variability and residual variability at this stage were estimated to be 37.6% and 48.8%, respectively.

Pharmacokinetic Models Incorporating Patient Covariates

In the models incorporating single covariates, when hematocrit and albumin were employed as discrete covariates, the OFV reduced significantly (Table 2, Fig. 4). However, when

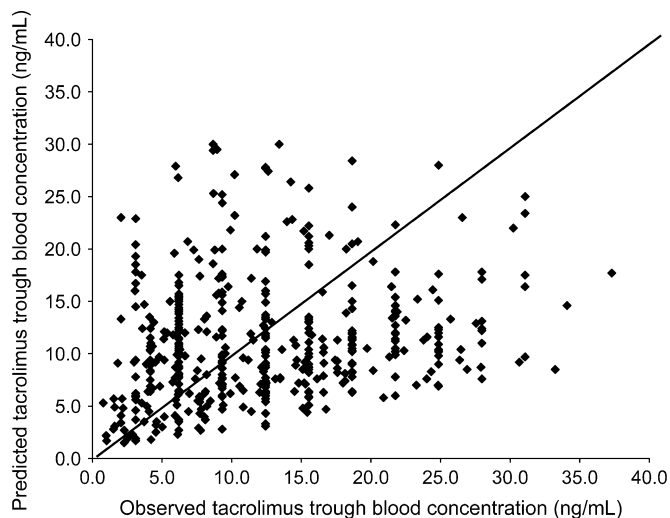


FIGURE 3. Base model 1 predicted versus observed tacrolimus trough blood concentration (ng/mL). Solid line represents line of unity ($n = 67$).

they were modeled as continuous covariates no such impact was observed. Patients with a low hematocrit (less than 35%) had a 46% higher apparent clearance than patients with >35% hematocrit levels. In patients with hypoalbuminemia (albumin level <3.5 g/dL), apparent clearance of tacrolimus was found to be 16% higher. The inclusion of serum total cholesterol concentration as a discrete covariate provided a significant decrease in OFV (Table 2), but the 95% confidence interval of the parameter estimate included the null value and hence was excluded from further analysis. Total protein concentration was not detected as a significant covariate when employed as either continuous or discrete variable.

Coadministration of CYP3A4 enzyme inhibitors, diltiazem ($n = 10$) and fluconazole ($n = 29$), were both detected as influential covariates in the single-covariate model analyses (Table 2, Fig. 4). However, phenytoin ($n = 11$) and omeprazole ($n = 35$) were not detected as significant covariates. In the exploratory analyses the impact of posttransplantation day as a significant covariate was evaluated as either a continuous or a discrete covariate (<30 days vs. >30 days posttransplantation). In accordance with the preliminary observations on change in the ratio of tacrolimus-daily-dose-to-trough-concentration over the posttransplantation period, apparent clearance of tacrolimus was found to decrease (by approximately 22%) from day 31 onward after the transplant. Inclusion of this factor as a discrete covariate into the base model yielded a significantly lower OFV (Table 2).

Demographic parameters (including patient's age, weight, gender, and ethnicity), clinical parameters such as liver function tests (ALT, AST, GGT, and bilirubin), diabetic/nondiabetic status, and the occurrence of allograft rejection were not detected as significant covariates.

Covariates included in the forward step of the model building included hematocrit, albumin concentration, and coadministration of fluconazole or diltiazem, all resulting in a significant decrease in OFV. Though posttransplantation day was detected to correlate with apparent clearance earlier, inclusion of this covariate into the combined model did not change OFV significantly.

In the final backward elimination step for the evaluation of covariates, a significant increase in OFV was observed on elimination of either hematocrit or albumin concentration and/or coadministration of fluconazole and diltiazem from the model. Therefore, these 4 covariates were included in the final population pharmacokinetic model (Table 3).

Model Evaluation

The quantitative evaluation (mean prediction error, ME; median prediction error, MdNE; root mean square error, RMSE; and mean absolute error, MAE) of the model performance using the validation dataset showed the model to be precise and free from bias (95% CI included zero). Estimates of ME and MdNE (measures of bias) using tacrolimus trough blood concentrations predictions from the final model and data from the validation dataset were small, and the 95% confidence interval included zero (ME -0.18 to 0.38 ng/mL; MdNE -0.15 to 0.40 ng/mL). The measures of precision (RMSE and MAE) associated with the validation group were small (RMSE 0.318 and MAE 1.96 ng/mL). Figure 5 presents the observed versus

TABLE 2. Results of Covariate Analyses Using Validation Dataset

Category of Covariate	Covariate in the Model	Covariate Scaling	ΔOFV	P Value
Demographic	Age	—	1	NS
	Weight	—	-2	NS
	Gender	—	4	NS
	Ethnicity	White = 1; others = 0	5	NS
Hematological and biochemical indices	Hematocrit*	Hematocrit <35% = 0; others = 1	-186	<0.01
	Albumin*	Albumin <3.5 g/dL = 0; others = 1	-71	<0.01
	Total cholesterol*##	Total cholesterol >5.2 mmol/L = 0; others = 1	-15	<0.01
	ALT*	ALT >55 U/L = 0; others = 1	-2	NS
	AST*	AST >55 U/L = 0; others = 1	-2	NS
	GGT*	GGT >55 U/L = 0; others = 1	0	NS
	Bilirubin	Bilirubin >18 μmol/L = 0; others = 1	-1	NS
Concurrent medication	Diltiazem†	Diltiazem coadministered = 0; others = 1	-80	<0.01
	Fluconazole‡	Fluconazole coadministered = 0; others = 1	-46	<0.01
	Occurrence of rejection§§	Rejection: yes = 0; no = 1	-2	NS
	Days posttransplant*	Days posttransplant >30 = 0; others = 1	-23	<0.01
	Diabetic/nondiabetic	Diabetic = 0, nondiabetic = 1	0	NS

*Covariates initially evaluated as continuous variable; however, ΔOFV was not statistically significant. NS, not significant.

##95% CI included negative value, so excluded from further analysis.

†Patients receiving diltiazem (n = 10) and ‡fluconazole (n = 29).

§Number of episodes of rejection (n = 33). SA 7-day window including 3 days before and after the occurrence of biopsy-proven rejection was considered as rejection period.

||Number of diabetic patients (n = 17).

predicted trough blood concentrations of tacrolimus, which shows a uniform distribution of points along the line of unity.

Finally, with the developed model population estimates of the parameters, interpatient and residual variability were also estimated for the complete dataset (Table 3). There was no significant change in the estimated population parameter values calculated using the total, index, and validation datasets

(Table 3). Based on the residual variability data, the model will have a prediction error of 2.7 ng/mL at a tacrolimus trough blood concentration of 11.1 ng/mL. With the complete dataset, the mean posterior Bayesian estimates of tacrolimus CL/F and V/F were 21.0 ± 8.4 L/h (range 4.3–43.9 L/h) and 316.1 L (range 84.1–789.1 L), respectively.

DISCUSSION

Tacrolimus whole-blood trough concentration is routinely monitored to optimize immunosuppressant therapy. However, the utility of monitoring this index to individualize tacrolimus therapy remains in question because of wide intra- and intersubject variability in tacrolimus pharmacokinetics and the lack of unequivocal evidence of an association between tacrolimus whole-blood trough concentration and clinical outcomes.^{18–20} Therefore, a detailed understanding of the factors contributing to the variability in tacrolimus pharmacokinetics may improve clinical use of this drug. In this study therapeutic drug-monitoring data for 67 liver transplant recipients were retrospectively collected, reviewed, and analyzed to provide estimates of tacrolimus CL/F and V/F using a population pharmacokinetic approach. A 1-compartment model with first-order absorption and elimination was used to describe tacrolimus concentration–time data. The FOCE method was used to allow residual error to be evaluated based on conditional parameter estimates rather than typical values.^{21,22} Furthermore, this approach has been reported to give better estimation when only trough concentrations are available for model development.²³ It was found that the patient’s hematocrit and serum albumin as well as coadministration of diltiazem and fluconazole had the most significant impact on tacrolimus apparent clearance.

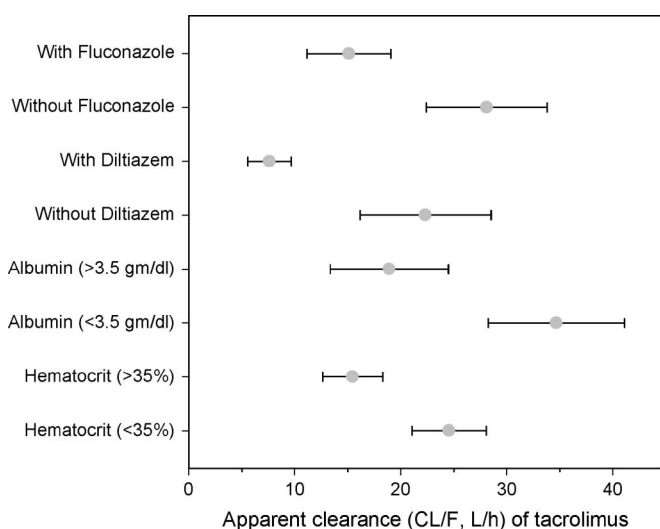


FIGURE 4. Effect of physiological state (anemia and hypoalbuminemia) and concurrent medications (fluconazole and diltiazem) on apparent clearance (L/h) of tacrolimus. Dots represent mean value, and error bars represent 95% confidence interval. CL/F was obtained using a univariate model in the base model.

TABLE 3. Population Parameter Estimates (95% CI) of the Pharmacokinetic Model Using Different Datasets

Parameters	Unit	Index Dataset (n = 47)	Validation Dataset (n = 20)	Whole Dataset (n = 67)
No. of observations	—	398	236	694
Apparent clearance (CL/F)	L/h	20.4 (16.9–23.9)	22.1 (19.1–25.1)	21.3 (18.0–24.6)
Apparent volume of distribution (V/F)	L	285.0 (110.0–460.0)	331.0 (176.0–486.0)	314.0 (133.0–495.0)
Absorption rate constant (k_a)	h^{-1}	4.5 (fixed)	4.5 (fixed)	4.5 (fixed)
Factor for hematocrit (θ_{HEM})	—	10.1 (7.8–12.4)	8.5 (7.1–9.9)	9.8 (7.4–12.2)
Factor for albumin (θ_{ALB})	—	3.1 (2.3–3.9)	3.8 (2.7–4.9)	3.4 (2.1–4.7)
Factor for diltiazem (θ_{DIL})	—	2.8 (1.7–3.9)	1.7 (1.2–3.2)	2.1 (1.4–2.8)
Factor for fluconazole (θ_{FLU})	—	8.9 (7.6–10.2)	6.6 (5.1–8.1)	7.4 (6.1–8.7)
Intersubject variability in CL/F	%	29.1	32.9	31.6
Residual random error	%	22.4	26.8	24.3

Model parameters:

$TVCL = \theta_{CL} + \theta_{HEM} \times (1 - HEM) + \theta_{ALB} (1 - ALB) - \theta_{DIL} \times (1 - DIL) - \theta_{FLU} * (1 - FLU)$.

$TVV = \theta_v$, $KA = \theta_{KA}$, $S2 = V$.

TVCL, typical (population) estimate of apparent clearance; TVV, typical (population) estimate of apparent volume of distribution.

HEM = 0 if hematocrit is less than 35%, otherwise 1.

ALB = 0, albumin is less than 3.5 g/dL, otherwise 1.

DIL = 0, diltiazem is co-administered, otherwise 1.

FLU = 0 if fluconazole is coadministered, otherwise 1.

The mean posterior Bayesian estimate of tacrolimus apparent clearance in this study was 21.0 L/h (range of individual Bayesian estimates of CL/F 4.3–43.9 L/h), which is in close agreement with the data reported by other researchers.^{2,4,16} Not surprisingly, this value was lower than the tacrolimus CL/F (44 L/h) reported in pediatric liver transplant recipients who received whole liver, and it was higher than the CL/F (5.75 L/h) observed in pediatric patients who received adult cut-down liver,⁶ which is a likely reflection of the lower metabolic activity in the cut-down graft. A moderate interpatient variability in apparent clearance (31.6%) was observed in the present population, which is similar to that reported in adult and pediatric liver transplant recipients.^{5,16,24} Reliable estimates of V/F could not be obtained in this analysis because the majority of tacrolimus concentration–time data were obtained

toward the end of the dosing interval. Therefore, in this analysis little emphasis was placed on estimates of V/F, and any possible covariate relationships were not explored.

Hypoalbuminemia and anemia are the 2 most common clinical conditions observed in liver transplant recipients, and both conditions were found to significantly increase tacrolimus CL/F in the cohort of patients included in this study. An increase in tacrolimus clearance with decreased hematocrit levels has been reported by other researchers.^{3,5} However, because of a lack of enough data points, these researchers could not quantify the impact of hematocrit on CL/F in their final population model. The impact of albumin concentration on tacrolimus CL/F is a unique and interesting finding of this study. Because it is a low- to intermediate-clearance drug with an unbound fraction of less than 1.0%, tacrolimus clearance may vary with variation in the extent of drug association with blood and plasma components.^{7,11} Therefore, the increase in CL/F in patients with anemia or hypoalbuminemia could be explained in part by an increase in the unbound fraction (caused by decrease in tacrolimus association with erythrocytes and plasma proteins). In fact, negative correlations between tacrolimus unbound fraction and patients' erythrocyte counts and plasma protein concentrations were also observed in our earlier cross-sectional study in liver transplant recipients.⁷

Like other researchers we have also observed a significant decrease in the tacrolimus dose (mg/d)-to-concentration (ng/mL) ratio (D/C) over the posttransplantation period (1.0 ± 0.5 to 0.7 ± 0.3).^{25–27} It is proposed that a decrease in tacrolimus dose-to-concentration ratio is associated with a decrease in clearance over the posttransplantation period. However, the mechanism of this is not known. Interestingly, at the later stage of the analysis in the full model, posttransplantation day was not selected as a significant covariate. It is likely that inclusion of the time-dependent covariates, including hematocrit and albumin concentrations, in the final model made use of posttransplantation days as a separate covariate for CL/F redundant.

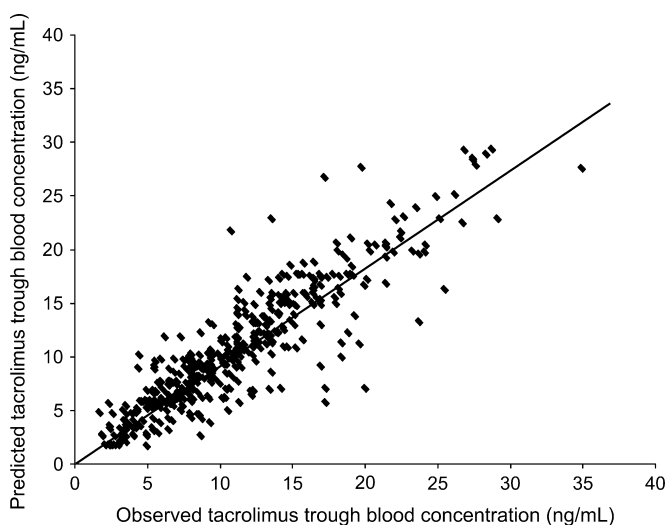


FIGURE 5. Final model predicted versus observed tacrolimus trough blood concentration (ng/mL). Solid line represents line of unity (n = 67).

Although it is recognized that the present study was not designed to investigate drug–drug interactions, significant interactions were observed between tacrolimus and the CYP3A4 enzyme inhibitors fluconazole and diltiazem. The drug interaction between tacrolimus and fluconazole was reported earlier in a conventional pharmacokinetic study in bone marrow transplant recipients.²⁸ Furthermore, a case of an increase in tacrolimus trough blood concentration after concurrent administration of diltiazem has been reported in liver transplant recipients.²⁹ In the present study, Bayesian estimates of CL/F showed an approximately 20% to 65% and 10% to 16% decrease in apparent clearance of tacrolimus when coadministered with fluconazole and diltiazem, respectively. This observation has significant clinical implications because these agents are often prescribed in transplant recipients, which reinforces the usefulness of blood level monitoring and application of population pharmacokinetic models as a tool in dose optimization of tacrolimus when coadministered with metabolic inhibitors.

In this study, despite a systematic investigation of the possible influence of covariates on the variability in tacrolimus apparent clearance, no association could be demonstrated with demographic characteristics, liver function test results, rejection episodes, and diabetic status of the patients. In contrast to the current findings, Staatz et al³ has reported a decrease in tacrolimus CL/F with an increase in hepatic enzymes (AST) in kidney transplant recipients and also in pediatric liver transplant recipients.⁶ These differences could be caused by a difference in the transplant population (liver versus kidney), age of the recipients (adult versus pediatric), and also time in the posttransplantation period (early versus late) investigated in the current study compared with other studies. In the current study most data were collected over the first 60 days after transplantation. Furthermore, after liver transplantation, the concentrations of liver enzymes (ALT, AST, GGT) remain considerably elevated for the first couple of weeks, which does not necessarily reflect poor graft function. This could explain the lack of an effect of liver function indices on tacrolimus CL/F in the current study.

Furthermore, an age-dependent change in tacrolimus CL/F was also reported by Sam et al (34% for every 1 year change in age from the median population age of 2.5 years across the age range of 1.1 to 13.9 years)⁵ and Staatz et al⁶ in pediatric liver transplant recipients. The possible explanation for this is the change in the metabolic function of the liver over the developmental phase of the child growth. In the present study all the patients were adults who received orthotopic liver transplant from adult donors; therefore, little age-dependent variation is expected in the metabolic activity of the transplanted liver.

In the current study, apparent clearance of tacrolimus was estimated using retrospectively collected therapeutic drug-monitoring data, which gave us an opportunity to assess the relative importance of various factors influencing tacrolimus disposition in liver transplant recipients in a clinical situation. Hypoalbuminemia, anemia, and concurrent medications including diltiazem and fluconazole were proven to be the most important covariates influencing tacrolimus CL/F. The population pharmacokinetics model developed in this study will allow transplant physicians to individualize tacrolimus dose

based on patient specific characteristics including albumin and hematocrit levels and administration of concomitant medications. Therefore, the use of the model developed in this study will provide clinicians with a tool to initialize tacrolimus dose and to change it more objectively, thereby eliminating the guesswork in dose individualization of this immunosuppressive agent. However, additional investigations are needed in a prospective study for comprehensive evaluation of the model in predicting tacrolimus concentration at different stages of the posttransplantation period.

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