

Tacrolimus in Diabetic Kidney Transplant Recipients: Pharmacokinetics and Application of a Limited Sampling Strategy

Anisha E. Mendonza, MSc,* Hamim Zahir, PhD,* Reginald Y. Gohh, MD,†
and Fatemeh Akhlaghi, PhD*

Abstract: The effect of diabetes mellitus on the pharmacokinetics of tacrolimus is not well characterized. We have compared tacrolimus 12-hour steady-state concentration-time profiles in diabetic (n = 11) and demographically matched nondiabetic (n = 9) stable kidney transplant recipients and derived a limited sampling strategy for the estimation of tacrolimus area under the concentration–time curve (AUC_{0–12}). Tacrolimus concentration was measured by liquid chromatography tandem mass spectrometry and acetaminophen absorption method was used to characterize gastric emptying time.

Demographic and biochemical characteristics were comparable between the two groups with the exception of significantly higher glycated hemoglobin levels in patients with diabetes ($P = 0.02$). Time to maximum concentration (T_{max}) of acetaminophen was significantly longer in diabetics [D: 74.1 minute versus nondiabetics (ND): 29.3 minutes, $P = 0.02$]; however, tacrolimus T_{max} was not significantly different (D: 121 minutes versus ND: 87 minutes, $P = 0.15$). Median (interquartile range) of tacrolimus AUC_{0–12} was 114 (101–161) $\mu\text{g}\cdot\text{hr}/\text{L}$ in patients with diabetes and 113 (87–189) $\mu\text{g}\cdot\text{hr}/\text{L}$ in nondiabetics ($P = 0.62$). The following limited sampling equation [$\text{AUC}_{\text{pred}} (\mu\text{g}\cdot\text{hr}/\text{L}) = 18.70 - 1.72 C_{1\text{hr}} - 4.09 C_{2\text{hr}} + 14.40 C_{3\text{hr}}$] was derived from a training data set that included 10 patients. The correlation coefficient between model-predicted and observed AUC_{0–12} values was 0.999. Mean prediction error and root mean square error of the model-predicted values derived from the patients in validation data set were 0.04 and 17.48 $\mu\text{g}\cdot\text{hr}/\text{L}$, respectively.

In conclusion, it appears that diabetes has a modest effect on the rate but not the extent of tacrolimus absorption, and a three-point abbreviated sampling strategy common to both groups may prove useful for the estimation of tacrolimus exposure in kidney transplant recipients.

Key Words: abbreviated AUC, diabetes, limited sampling strategy, pharmacokinetics, tacrolimus

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From the *Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, Rhode Island; and †Division of Organ Transplantation, Rhode Island Hospital, Brown University Medical School, Providence, Rhode Island.

Correspondence: Fatemeh Akhlaghi, PhD, Biomedical and Pharmaceutical Sciences, University of Rhode Island, 125 Fogarty Hall, 41 Lower College Road, Kingston RI 02881 (e-mail: fatemeh@uri.edu).

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Tacrolimus (Prograf; Astellas Pharma US Inc., Deerfield, IL) is a calcineurin inhibitor that is frequently used to prevent rejection in solid organ transplantation in combination therapy with mycophenolic acid and prednisone.¹ Tacrolimus was introduced in the 1990s and has a mechanism of action similar to cyclosporine but it is more potent than cyclosporine.² A combination triple therapy with tacrolimus, mycophenolic acid, and prednisone has been shown to reduce the rate of acute rejections in highly sensitized kidney allograft patients.³

Tacrolimus exhibits a narrow therapeutic index and marked intra- and interindividual variability in its pharmacokinetic characteristics.⁴ It is therefore difficult to predict the concentrations achieved with a nominal tacrolimus dose in a given patient necessitating routine therapeutic drug monitoring. The recommended starting oral dose of tacrolimus after kidney transplantation ranges between 0.2 and 0.3 mg/kg per day in two divided doses⁵ followed by dose adjustment to achieve a predefined trough concentration (C_{trough} or C_{min}) suitable for the type of transplant and the time after transplantation.

Factors affecting the pharmacokinetic characteristics of tacrolimus include type of transplant, time posttransplant, race, age, hepatic function, concomitant medications (namely coadministration of CYP3A4 inhibitors), and the degree of expression of efflux transporters, p-glycoproteins (MDR-1), in the intestine.^{4,6,7} The bioavailability of tacrolimus is approximately 25% with a reported range of 5% to 93%.⁸ Tacrolimus bioavailability is altered because of several pathophysiological conditions altering gastrointestinal motility. Among those, increased rate and extent of tacrolimus absorption was observed in patients with persistent diarrhea⁹ or concomitant administration of metoclopramide.¹⁰ Considering this, it is conceivable to believe that tacrolimus pharmacokinetics may be altered in patients with reduced gastric emptying time secondary to long-term diabetes mellitus.

According to the U.S. Renal Data System annual data report, more than 30% of kidney transplant recipients in the United States have preexisting type 1 or 2 diabetes¹¹ with an additional 10% developing diabetes after transplantation.¹² Diabetes mellitus can potentially alter the pharmacokinetics and/or pharmacodynamics of many drugs.¹³ Diabetes may affect the autonomic nervous system causing neuropathy and consequently alter the motility of the gastric muscles resulting in delayed gastric emptying and gastroparesis.¹⁴ The delay in gastric emptying time can theoretically slow the oral absorption of drugs, therefore affecting pharmacokinetic

parameters, including T_{\max} , maximum concentration (C_{\max}), or AUC.

The pharmacokinetics of tacrolimus in diabetic organ transplant recipients has not been characterized previously. Two short reports have been published to date^{15,16} describing the pharmacokinetic characteristics of tacrolimus in patients with diabetes with end-stage renal dysfunction awaiting kidney transplantation. The objective of the present study was to assess tacrolimus pharmacokinetics in diabetic stable kidney transplant recipients in comparison with demographically matched nondiabetics and to devise a limited sampling strategy for the estimation of tacrolimus AUC common to both diabetics and nondiabetics.

MATERIALS AND METHODS

Study Design and Patient Population

Twenty stable kidney transplant recipients, aged 18 to 65 years, were included in the study. Eleven patients were known to be long-term diabetic (type 1 or 2) and nine patients had no evidence of diabetes mellitus. Study protocol was approved by the Institutional Review Board (IRB #0159-03 and 0054-05) of Rhode Island Hospital, Providence, Rhode Island. Patients were recruited by kidney transplant physicians at Rhode Island Hospital and before the study, the procedure was explained to the patients and their signed informed consent was obtained.

The immunosuppressive protocol included triple therapy with tacrolimus, prednisone, and mycophenolic acid administered either as mycophenolate mofetil (11 patients) or enteric-coated mycophenolate sodium (nine patients). Other concomitant medications commonly prescribed included antibacterial, antiviral, antihypertensive, lipid-lowering, antiplatelet, and cardiovascular agents. Generally, the patients with diabetes were matched with nondiabetics on the basis of gender, approximate age, race, time posttransplant, mycophenolic acid dose, and kidney function (± 10 mL/min estimated creatinine clearance); however, despite best efforts, complete matching could not be achieved in every category. Excluded were patients who were pregnant or nursing and those who had received a pancreatic transplant.

Pharmacokinetics Study

Patients had fasted from the previous night and arrived at the study center at 7:00 AM. On the study day, patients underwent a physical examination that included blood pressure measurement, weight, height, and urinalysis. Patients then took their morning medications, including immunosuppressive agents (at approximately 8:00 AM) and were also given 1000 mg acetaminophen (Concentrated Tylenol Infants' Drops; McNeil Consumer and Specialty Pharmaceuticals, Washington, PA) that served as a gastric emptying rate marker. All the medications were consumed with 250 mL water. Blood samples were collected into K2-EDTA Vacutainers (Beckton Dickinson; Franklin Lakes, NJ) before dose and at 0.25, 0.5, 1, 1.5, 2, 3, 5, 7, 9, 10, and 12 hours postdose. Generally, patients continued fasting for 2 hours after dosing but were allowed to consume clear liquids, including a fruit

juice-based nutritional drink (Boost Breeze; Novartis, Basel, Switzerland) at any time to prevent hypoglycemia. All patients were given standardized diabetic hospital meals (comprising a total of 2000 Kcal per day) at approximately 10:00 AM, 1:00 PM, and 7:00 PM.

Analytic Methods

All assays were performed at the Clinical Pharmacokinetics Research Laboratory, University of Rhode Island, using in-house validated assays. Acetaminophen concentrations were measured in plasma samples up to 5 hours postdose using a previously described high-performance liquid chromatography method.¹⁷ Tacrolimus concentrations were measured as described in the following section using a liquid chromatography–tandem mass spectrometry method.¹⁸ Both assays were validated according to the guidelines set by the U.S. Food and Drug Administration.¹⁹ Concentration of glycated albumin (glycoalbumin) in plasma samples were measured according to the manufacturer's instruction by an enzyme-linked immunosorbent assay using Glycaben kit (Exocell, Philadelphia, PA).

Analysis of Tacrolimus

Tacrolimus and the internal standard ascomycin were purchased from LC Laboratories (Woburn, MA). All other reagents were high-performance liquid chromatography grade and were purchased from Fisher Scientific (Hampton, NH). Briefly, 0.5 mL of a patient sample or calibrator (EDTA anticoagulated whole blood) was pipetted into 1 mL of a precipitating reagent, acetonitrile containing 50 μ g/L of ascomycin. Samples were vortex mixed for 1 minute and centrifuged at 4°C 860 g for 5 minutes. Supernatants were subjected to solid-phase extraction. Sep-Pak C₁₈ solid-phase extraction cartridges (200 mg, 3 mL capacity; Waters, Milford, MA) were assembled on a Visiprep DL vacuum manifold (Supelco, Bellefonte, PA) and preconditioned with methanol (6 mL) and deionized water (6 mL). The cartridges were washed with 6 mL deionized water, followed by methanol: water (50:50 v/v, 3 mL) and heptane (2 mL), dried for 15 minutes under vacuum, and the analytes were eluted with 1:1 mixture of isopropanol and heptane (2.5 mL). Thereafter, the eluent was dried in a centrifugal evaporator (SPD1010 Speed Vac; ThermoSavant, Waltham, MA) at 60°C for approximately 50 minutes. The dried samples were reconstituted in 0.25 mL absolute methanol and 0.1 mL of the reconstituted sample was injected onto the analytic column.

Liquid Chromatography–Tandem Mass Spectrometry Instrumentation

A liquid chromatography system comprising of autosampler and micropumps (Perkin Elmer, Norwalk, CT) coupled with an ABI-Sciex 2000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) was used for tacrolimus quantification. Tacrolimus and ascomycin were detected in the positive ion mode using the mass transitions m/z 821.5 \rightarrow 768.1 for tacrolimus and m/z 809.1 \rightarrow 756.1 for ascomycin. Analytical column was an Aquaperfect (150 mm \times 3.0 mm, 5 μ M) C₁₈ reverse-phase

column (MZ Analysentechnik, Mainz, Germany). Elution of the analytes from the analytic column was carried out using a gradient mobile phase composition comprising of 80:20% (v/v) methanol: 30 mmol/L ammonium acetate (1 minute; 0.25 mL/min), switching to a 97:3% (v/v) methanol: 30 mmol/L ammonium acetate (4.5 minutes; 0.35 mL/min) and changing back to the 80:20 composition for the last 1.5 minute (0.25 mL/min).

Assay Validation

The lower limit of quantification for tacrolimus was 0.5 µg/L and the calibration curve ranged from 0.5 to 100 µg/L ($r^2 = 0.998$). The absolute recovery for tacrolimus and ascomycin ranged between 88% to 96% and 89% to 97%, respectively. The intra- and interday percent coefficient of variation for the quality control (QC) samples at 3 µg/L, 15 µg/L, and 30 µg/L concentrations ranged from 1.5% to 1.7% and 6.9% to 9.9%, respectively. The inaccuracy for the method was $5.4\% \pm 1.1\%$ for 3 µg/L QC, $4.4\% \pm 2.0\%$ for 15 µg/L QC, and $3.6\% \pm 1.7\%$ for 30 µg/L QC samples.

Pharmacokinetic Analysis

The concentration–time data were analyzed using WinNonLin version 5.0.1 (Pharsight Corp., Mountain View, CA). A noncompartment model with extravascular input for blood data at steady state was used to obtain estimates of the pharmacokinetic parameters of tacrolimus in blood, including C_{max} , T_{max} , AUC_{0-12} , and apparent clearance at steady state. The AUC_{0-12} indicates the area under the concentration–time curve from the first time point of blood collection (0 hour) to the last time point (12 hours) and was calculated by the linear trapezoidal rule. Also, as a result of the presence of an apparent secondary peak in the elimination phase of tacrolimus, observed in the concentration–time profiles of certain patients, no attempt was made to calculate tacrolimus elimination rate constant or half-life as well as volume of distribution at steady state. Instead mean residence time (MRT), the average time a given drug molecule resides in the body, was calculated using the ratio of the area under the first moment curve and the AUC_{0-12} . Percentage of fluctuation (%fluctuation) in concentration was calculated using the following equation implemented in WinNonLin software: $(C_{max} - C_{min})/C_{avg} * 100$, where C_{avg} is $AUC_{0-12}/12$.

Statistical Analysis

All statistics were performed using SPSS 13.0 (SPSS Inc., Chicago, IL) to compare the differences in tacrolimus pharmacokinetic parameters between diabetic and nondiabetic patients. Kolmogorov-Smirnov test with the Lilliefors correction was used to test whether data follows Gaussian distribution. Unless otherwise stated, all normally distributed data were expressed as mean \pm standard deviation or median or interquartile range if nonnormally distributed. An independent samples t test was used to compare normally distributed data and the Mann-Whitney U test was used for comparisons of nonnormally distributed data. All statistical significance was based on a P value of 0.05. Pharmacokinetic

parameters were also compared when patients were divided into two groups based on the glucose control over the last 2 to 3 months as judged by glycated hemoglobin (HbA1c) levels [6.5% or less (nine patients; two D, seven ND) versus greater than 6.5% (11 patients; nine D, two ND)].²⁰

Limited Sampling Strategy

A limited sampling strategy was used to establish an abbreviated AUC equation. Patient data were randomly divided into two groups [training data set ($n = 10$) and validation data set ($n = 10$)] using the random selection process in SPSS. Both groups contained an equal number of patients with diabetes. From the training data set, the tacrolimus concentration for each sampling time point was correlated with the measured tacrolimus AUC_{0-12} . A stepwise linear regression method was then applied to the tacrolimus concentrations up to 3 hours postdose as independent variables and tacrolimus AUC_{0-12} as the dependent variable.

The equations generated postregression were validated using the validation data set. Validation included assessing the predictive performance of the equations using the method described by Sheiner and Beal²¹ that included determination of mean prediction error (MPE) and the mean percentage of the prediction error (MPPE) as measures of bias and root mean squared prediction error (RMSE) as a measure of precision using the following equations:

$$MPE = (1/n) \sum_{i=1}^n (AUC_{pred} - AUC_{all})$$

$$MPPE = (1/n) \sum_{i=1}^n 100(AUC_{pred} - AUC_{all})(1/AUC_{all})$$

$$RMSE = \sqrt{(1/n) \sum_{i=1}^n (AUC_{pred} - AUC_{all})^2}$$

Predictive performance of the equation was also illustrated using Bland and Altman plot.²² The Bland and Altman plot provides simple graphic representation of agreement between the two methods of measurement.

RESULTS

Demographic characteristics, biochemical indices, and doses of immunosuppressive agents are presented in Table 1. The etiology of end-stage kidney disease was diabetic nephropathy ($n = 9$), IgA nephropathy ($n = 3$), polycystic kidney disease ($n = 4$), focal segmental glomerulosclerosis ($n = 1$), hypertension ($n = 1$), hypertensive nephrosclerosis ($n = 1$), and congenital urologic dysplasia ($n = 1$). Generally, both groups had comparable demographic characteristics and biochemical indices with the exception of significantly higher HbA1c values in patients with diabetes (Table 1). There were no statistically significant differences between the doses of mycophenolic acid or prednisone between patients with diabetes and nondiabetic patients; however, tacrolimus daily dose was marginally lower ($P = 0.07$) in patients with diabetes.

In addition to immunosuppressive agents, 20 other medications were taken by the patients with diabetes in addition to antidiabetic therapy (insulin, pioglitazone, glipizide) as compared with eight medications taken by nondiabetic

TABLE 1. Demographic Characteristics of the Patient Population (Values are Presented as Median and Interquartile Range)

	Diabetic (n = 11)	Nondiabetic (n = 9)	P Values
Age (years)	57 (50–62)	39 (18–53)	0.14
Weight (kg)	82 (71–96)	84 (74–100)	0.93
Time posttransplant (months)	15 (10–30)	14 (8–32)	0.63
Tacrolimus daily dose (mg)	5 (3–6)	5 (4.5–13)	0.07
Tacrolimus morning dose (mg)	3 (1.5–3)	3 (2–6.5)	0.08
MPA morning dose (mg)	Cellcept (n = 11)	500 (500–500)	0.72
	Myfortic (n = 9)	360 (360–630)	0.68
Prednisone dose (mg/day)	5 (5–10)	5 (5–9.5)	0.60
Glycated hemoglobin (%)	7.1 (6.6–8.6)	5.6 (4.8–6.5)	0.02
Glucose (mg/dL)	128 (84–152)	94 (83–119)	0.23
Albumin (mg/dL)	4.2 (4.0–4.4)	4.3 (4.2–4.6)	0.27
Glycated albumin (%)	1.3 (1.0–1.6)	1.1 (0.8–1.4)	0.25
Cholesterol (mg/dL)	182 (159–196)	165 (139–203)	0.59
Low-density lipoprotein cholesterol (mg/dL)	113 (88–124)	84 (64–112)	0.18
High-density lipoprotein cholesterol (mg/dL)	50 (25–75)	55 (38–63)	0.13
Triglyceride (mg/dL)	140 (110–183)	178 (157–280)	0.09

patients. The additional medications included antihyperlipidemics (ezetimibe, simvastatin), cardiovascular agents (clonidine, amlodipine besylate/atorvastatin calcium), antiulcer agents (omeprazole, ranitidine), antifungal agents (clotrimazole), and antidepressants (bupropion).

Delayed gastric emptying was more pronounced in patients with diabetes versus nondiabetics as confirmed by the results of the acetaminophen concentration–time profiles. Acetaminophen T_{max} was significantly longer (D: 74.1 ± 57.0 minutes versus ND: 29.3 ± 11.3 minutes, $P = 0.02$) and C_{max} was lower (D: 12.6 ± 4.0 mg/L versus ND: 16.3 ± 4.0 mg/L, $P = 0.048$). Acetaminophen area under the concentration–time curve (AUC_{0-5}) was not significantly different ($P = 0.450$) between the two groups.

Figure 1 depicts the individual concentration–time profiles of tacrolimus in patients with diabetes (Fig. 1A) and nondiabetics (Fig. 1B) and Figure 2 represents average tacrolimus blood concentration–time profiles in the two groups.

Tacrolimus pharmacokinetic parameters are presented in Table 2. Daily dose of tacrolimus was marginally lower in patients with diabetes (Table 1); therefore, all pharmacokinetic parameters are expressed as both nondose-normalized and dose-normalized values. There were no significant differences between any of the parameters between patients with diabetes and nondiabetics. On average, tacrolimus T_{max} was delayed by approximately 30 minutes in patients with diabetes; however, the difference was not statistically significant between the two groups ($P = 0.15$). The acetaminophen T_{max} correlated well with tacrolimus T_{max} in patients with diabetes ($r = 0.71$, $P = 0.014$) but not in nondiabetic patients ($r = -0.52$, $P = 0.155$). A review of the individual concentration–time profiles (Fig. 1) reveals that one diabetic profile had higher C_{min} and C_{max} values than the remainder of the patients with diabetes. However, this patient had poor diabetic control (HbA1c = 9.5%), was on a higher tacrolimus dose when compared with the remainder of patients with diabetes, and was taking omeprazole, a known CYP3A inhibitor.⁷

Dividing patients into two groups based on the HbA1c values 6.5 or less versus greater than 6.5% based on the guidelines set by the American Association of Clinical Endocrinologists²⁰ revealed no significant differences in most of the pharmacokinetic parameters of tacrolimus, including AUC_{0-12} or C_{max} . The only pharmacokinetic parameter that was significantly different was MRT: 5.3 ± 0.39 hours in patients with HbA1c greater than 6.5 and 5.7 ± 0.43 hours ($P = 0.036$) in patients with adequate glucose control (HbA1c 6.5 or less).

Limited Sampling Strategy Results

There were no significant differences in the demographic characteristics of patients included in the training or validation data sets. Pharmacokinetic parameters, including AUC_{0-12} , C_{max} , T_{max} , C_{min} (morning), and C_{min} (evening), were not different (data not shown). Table 3 gives the coefficient of determination (r^2) for tacrolimus AUC_{0-12} values and concentrations at each sampling time point for the training data set. The best correlation was observed for the concentration at 10 hours postdose ($r^2 = 0.949$). All other time points also correlated significantly with the AUC_{0-12} . However, to derive a pragmatic limited sampling equation, we have only included the concentration–time data from 0 to 3 hours (0, 0.25, 0.5, 1, 1.5, 2, and 3 hours) into the stepwise multiple linear regression equation. This produced three equations with the third equation that presented a correlation coefficient (r) of 0.999 between AUC_{0-12} and AUC_{pred} for the training data set (Table 4).

The three equations were tested for their predictive performance using the validation data set. The predictive performance of the equations is shown in Table 5. The MPE for equation 3 was not significantly different from zero indicating that the predictions were not biased. Also, the MPPE was 0.84%. The Bland and Altman plot presented in Figure 3 effectively represents adequate agreement and lack of bias between model-predicted and observed AUC_{0-12} values.

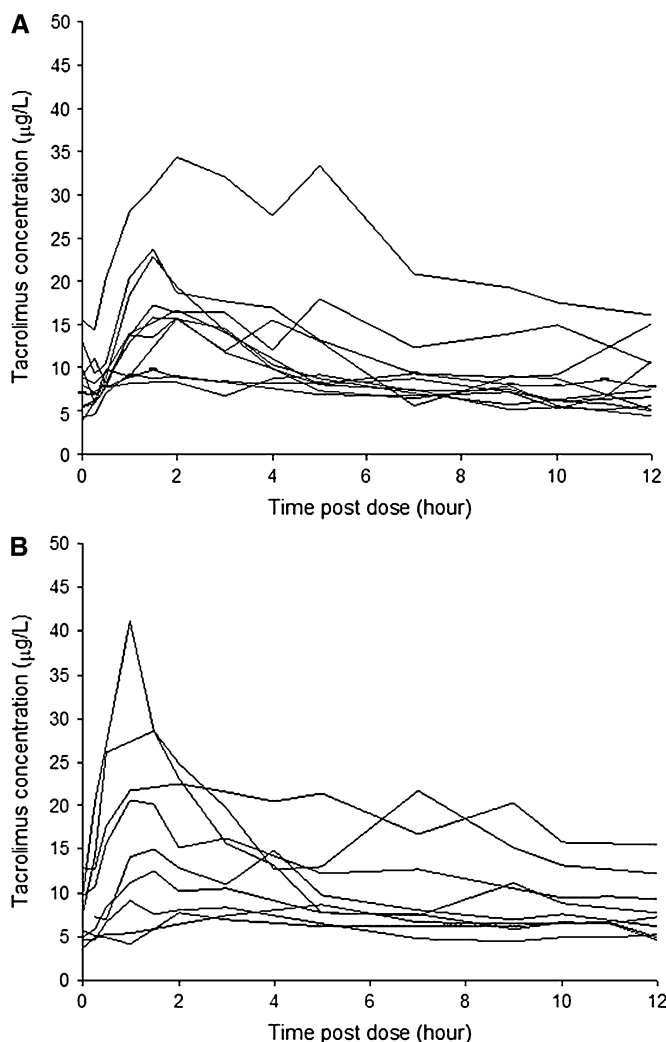


FIGURE 1. Individual tacrolimus concentration–time profiles for patients with diabetes (A, n = 11) and nondiabetic (B, n = 9) patients.

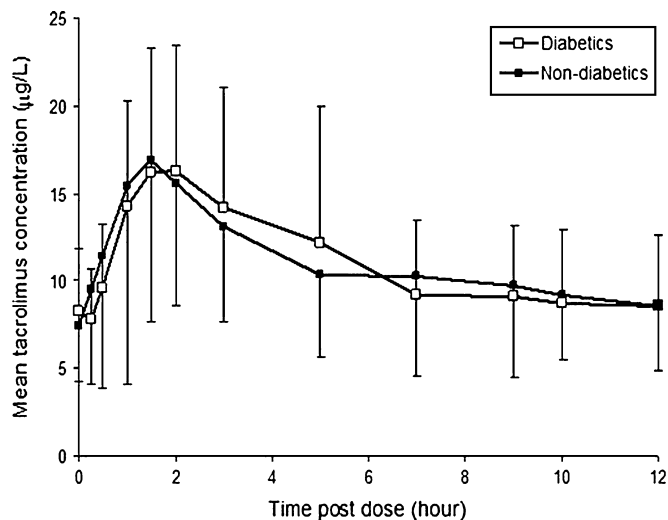


FIGURE 2. Mean tacrolimus blood concentration–time profile in patients with diabetes (n = 11) and nondiabetic (n = 9) patients (error bars represent standard deviation).

DISCUSSION

In many countries, including the United States, diabetes mellitus has become the most frequent cause of end-stage kidney disease requiring renal replacement therapy that may include kidney transplantation.²⁵ The pathophysiological alterations associated with diabetes may affect the pharmacokinetics of drugs including drug absorption, distribution, metabolism, and elimination. To date, no systematic evaluation of tacrolimus pharmacokinetics in diabetes has been reported in stable transplant recipients. However, one study that investigated single-dose tacrolimus pharmacokinetics in diabetic and nondiabetic individuals awaiting kidney and/or combined kidney–pancreas transplants reported tacrolimus AUC to be 38% lower in patients with diabetes.¹⁶

We have investigated tacrolimus pharmacokinetics in stable kidney transplant recipients with a median of 15 months

TABLE 2. Comparison of the Pharmacokinetic Parameters of Tacrolimus Between Diabetic (n = 11) and Nondiabetic (n = 9) Stable Kidney Transplant Recipients

Pharmacokinetic Parameters	Nondose-Normalized			Dose-Normalized*		
	Diabetic	Nondiabetic	P Value	Diabetic	Nondiabetic	P Value
Time to maximum concentration (minutes)	121 (91–131)	87 (68–126)	0.15			
C ₀ (µg/L)†	8.1 (5.5–10.1)	6.2 (4.8–9.7)	0.57	3.9 (2.1–5.1)	2.1 (1.3–3.7)	0.10
C ₁₂ (µg/L)‡	7.5 (4.4–16.1)	7.3 (4.7–15.5)	0.85	4.0 (1.9–5.4)	2.4 (1.5–3.7)	0.12
C _{max} (µg/L)	15.8 (9.1–34.4)	15.1 (7.8–41.2)	0.62	6.1 (5.6–8.6)	4.8 (4.8–6.7)	0.07
Area under the concentration–time curve (µg*hr/L)	114 (101–161)	113 (87–189)	0.62	55 (38–73)	37 (28–59)	0.07
Apparent clearance (L/hr/kg)	0.25 (0.14–0.36)	0.34 (0.20–0.47)	0.27			
Mean residence time (hours)	5.4 (5–5.8)	5.4 (5.2–5.9)	0.62			
Fluctuation index (%)	100 (70–113)	87 (59–134)	0.73			

All values are expressed as median (interquartile range).

*Dose-normalized pharmacokinetic parameters have been normalized based on the respective dose of tacrolimus and also multiplied by 1000.

†C₀: trough concentration of tacrolimus observed in the morning.

‡C₁₂: trough concentration of tacrolimus observed in the evening.

TABLE 3. Tacrolimus Concentrations at Different Times Postdose and the Coefficient of Determination (r^2) With AUC_{0-12} for the Patients Included in Training Data Set ($n = 10$)*

Time Postdose (Hours)	Tacrolimus Concentration ($\mu\text{g/L}$)		Coefficient of Determination (r^2)
	Median	Interquartile Range	
0	7.7	4.2–13	0.814
0.25	7.8	5–13.2	0.743
0.5	8.9	6.2–16.7	0.909
1	19.2	8.4–23.3	0.661
1.5	21.6	10–27.4	0.778
2	18.7	12.1–23.7	0.853
3	15.3	8.1–20.2	0.941
5	10.4	7.2–15.2	0.840
7	8.4	6.7–17.8	0.880
9	8.4	6.5–16.2	0.895
10	9.2	5.5–14.5	0.949
12	10.8	5.1–15.3	0.681

AUC, area under the concentration–time curve.

*The concentrations at each time point correlated significantly with the AUC_{0-12} ($P < 0.001$).

posttransplant. All patients with diabetes were matched with nondiabetics so at the completion of the study, with the exception of HbA1c levels, most demographic or biochemical characteristics were comparable between the two groups. In this study, all primary pharmacokinetic parameters of tacrolimus, including C_{max} or AUC_{0-12} , were comparable between diabetic and nondiabetic patients with the exception of tacrolimus T_{max} that was marginally but not significantly delayed.

Long-term diabetic patients typically have delayed gastric emptying,^{24,25} which affects the rate of absorption of some drugs. We have characterized gastric emptying rate by measuring the rate of absorption of acetaminophen. Acetaminophen is a basic compound and is absorbed exclusively after reaching the small intestine; therefore, the rate of appearance of acetaminophen in the systemic circulation represents the rate of gastric emptying.²⁶ Acetaminophen T_{max} was significantly longer and its C_{max} was lower in patients with diabetes. Despite this delay, tacrolimus T_{max} was marginally delayed and tacrolimus C_{max} was not different between the two groups. Our result is consistent with Kuypers et al, who observed that delayed gastric emptying affected the rate but not the extent of tacrolimus absorption.²⁷

Also, both the rate and extent of tacrolimus absorption was reduced because of concurrent administration with a moderate fat content meal.²⁸ In this study, all patients consumed their first meal approximately 2 hours after tacrolimus dose so the absorption was not affected by food ingestion. Also, none of our patients were taking metoclopramide that is known to increase the rate of tacrolimus absorption.¹⁰ Inspecting individual concentration–time profiles of tacrolimus shows prolonged absorption phase and flat absorption profiles in both groups, which may explain a broad range of values observed for tacrolimus T_{max} resulting in the lack of statistical significance. Also, an apparent secondary peak was observed in the tacrolimus concentration–time profiles from certain individuals, which may be the result of prolonged and inconsistent absorption, indistinguishable peak concentration, or other unknown phenomenon. A post hoc sample size calculation revealed that given the large degree of variability in tacrolimus absorption rate, a sample size of 89 patients in each group would be needed to achieve a P value of less than 0.05 with 80% power. Our study was therefore grossly underpowered to detect a statistical significance in tacrolimus T_{max} between the two groups.

Diabetes may also influence protein binding of drugs because of elevated levels of glycosylated proteins, including albumin or hemoglobin, thereby affecting the clearance of restrictively cleared drugs.²⁹ Tacrolimus is highly bound to red blood cells and is 99% bound to plasma proteins leaving a small unbound fraction.³⁰ A difference in drug binding may be evident through unbound fraction determination, which was not estimated in this study. The concentration of binding proteins, including cholesterol, serum lipids, and albumin (also glycosylated albumin), was not significantly different between the two groups. Because of an apparent secondary peak in the tacrolimus elimination phase, we have not been able to calculate values of terminal elimination rate constant or half-life. Although the values of MRT were significantly lower in patients with poor glucose control (HbA1c greater than 6.5%), however, little can be concluded from such a small difference in the MRT values between the two groups. Also, it must be noted that MRT values after oral administration can be misleading as an indicator for drug elimination because it reflects both the absorption and elimination phases.

The overall exposure to tacrolimus as judged by nondose-normalized AUC values was highly comparable between diabetic and nondiabetic patients. Tacrolimus daily dose was marginally lower in patients with diabetes resulting in borderline higher dose-normalized AUC or C_{max} values and

TABLE 4. Stepwise Regression Equations Generated After Input of Tacrolimus Concentrations From 0 to 3 Hours Postdose

Equations	Time Points	Regression Equations	Correlation Coefficient (r)	P
1	$C_{3\text{hr}}$	$AUC_{\text{pred}} = 7.7 + 8.7 C_3$	0.990	<0.01
2	$C_{2\text{hr}}, C_{3\text{hr}}$	$AUC_{\text{pred}} = 13.9 - 4.3 C_{2\text{hr}} + 13.1 C_{3\text{hr}}$	0.997	<0.01
3	$C_{1\text{hr}}, C_{2\text{hr}}, C_{3\text{hr}}$	$AUC_{\text{pred}} = 18.7 - 1.7 C_{1\text{hr}} - 4.1 C_{2\text{hr}} + 14.4 C_{3\text{hr}}$	0.999	<0.01

The P value represents the significance of the association between the predicted AUC using derived equation and the observed AUC_{0-12} .
C, concentration; AUC, area under the concentration–time curve.

TABLE 5. The Predictive Performance of the Limited Sampling Strategy Applied to the Three Equations and Calculated Using the Validation Data Set (n = 10)*

	Equation 1	Equation 2	Equation 3
Mean prediction error (µg*hr/L)	-3.3 (-13.5, 6.9)	-3.4 (-15.2, 8.3)	0.04 (-11.4, 11.4)
Mean percentage of the prediction error (%)	-2.7 (-12.3, 6.9)	-1.9 (-13.2, 9.6)	0.89 (-10.4, 12.2)
Root mean squared prediction error (µg*hr/L)	15.90 (13.4, 18.4)	18.3 (14.7, 21.9)	17.48 (16.8, 18.2)

Equation 1: $AUC_{pred} = 7.7 + 8.7 C_3$.

Equation 2: $AUC_{pred} = 13.9 - 4.3 C_{2hr} + 13.1 C_{3hr}$.

Equation 3: $AUC_{pred} = 18.7 - 1.7 C_{1hr} - 4.1 C_{2hr} + 14.4 C_{3hr}$.

*The values represent the mean (95% confidence intervals).

lower apparent clearance. Tacrolimus dose is adjusted clinically based on routine trough concentration monitoring. The reduced daily dose requirement for tacrolimus in patients with diabetes is an interesting observation that requires further evaluation in the context of a longitudinal study in a larger number of patients. It can be speculated that patients with diabetes either have reduced intestinal CYP3A4 or MDR-1 activity or are administered CYP3A4 inhibitors⁷ more frequently resulting in lower doses of tacrolimus required to achieve the same blood concentrations.

Trough concentration monitoring is currently used as a guide to tacrolimus dose adjustment.³¹ However, episodes of rejection or toxicity may occur despite adequate trough concentrations of tacrolimus⁶ suggesting that measurement of total exposure to tacrolimus may be a better indicator for the drug effect. Measuring drug AUC may provide a more accurate measure of total drug exposure and, at least in

theory, an indicator of drug effectiveness.³² The traditional method to measure AUC includes measuring concentrations at six or more time points, which is not routinely practical. To date, there have been at least four studies that have investigated abbreviated AUC monitoring and limited sampling strategy for tacrolimus monitoring in kidney transplantation aiming to predict the AUC.³²⁻³⁶ In all these strategies, tacrolimus concentrations were measured using immunoassays. We estimated whole blood concentrations of tacrolimus using an in-house validated liquid chromatography-tandem mass spectrometry method, which was highly specific and sensitive for tacrolimus.

Because the major pharmacokinetic parameters were not different between patients with diabetes and nondiabetics, we aimed to establish a pragmatic limited sampling approach common to both groups. A previous study established an equation with sample collection times up to 4 hours postdose.³³ The limited sampling approach in this study aimed to include blood collection time points up to 3 hours to obtain AUC_{pred} with the best possible association to the AUC_{0-12} . A 3-hour period would make it convenient to recruit transplant patients for clinical studies with transplant patients in which an estimate of the tacrolimus AUC_{0-12} is desired. Although later time points (9, 10, and 12 hours postdose) generated adequate prediction equations, inclusion of these time points would defy the purpose; therefore, such equations were not further pursued. Also, predictive performance of the final model was adequate indicating lack of systematic imprecision or bias. This limited sampling method may be useful for clinicians or researchers interested in the estimation of tacrolimus AUC without the need to collect blood samples for 12 hours postdose.

CONCLUSION

Diabetes appears to have minimal effect on the rate and no effect on the extent of absorption of tacrolimus in stable kidney transplant recipients; however, the influence of diabetes on tacrolimus apparent clearance or dose requirement must be studied in a longitudinal study with a larger number of patients.

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REFERENCES

1. Taylor AL, Watson CJ, Bradley JA. Immunosuppressive agents in solid organ transplantation: mechanisms of action and therapeutic efficacy. *Crit Rev Oncol Hematol.* 2005;56:23-46.
2. Jiang H, Sugu H, Takahara S, et al. Combined immunosuppressive effect of FK 506 and other immunosuppressive agents on PHA- and CD3-stimulated human lymphocyte proliferation in vitro. *Transplant Proc.* 1991;23:2933-2936.
3. Gonwa T, Johnson C, Ahsan N, et al. Randomized trial of tacrolimus + mycophenolate mofetil or azathioprine versus cyclosporine + mycophenolate mofetil after cadaveric kidney transplantation: results at three years. *Transplantation.* 2003;75:2048-2053.
4. Venkataraman R, Swaminathan A, Prasad T, et al. Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokinet.* 1995;29:404-430.

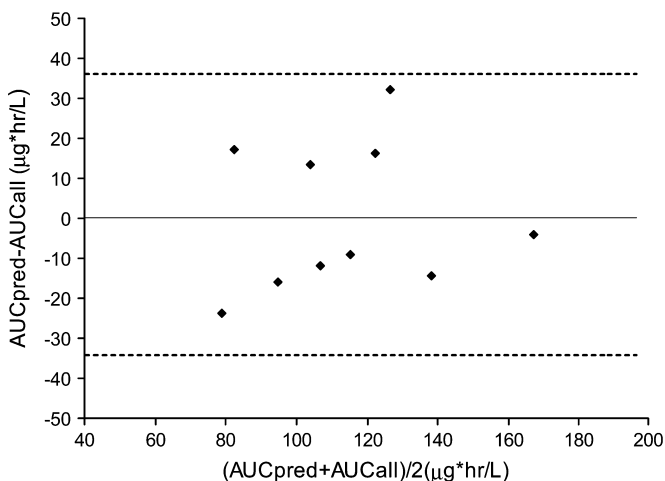


FIGURE 3. Bland and Altman plot for the agreement between measured AUC_{0-12} and estimated AUC_{pred} for validation data set. The solid line represents the mean bias; dotted lines presented ± 2 standard deviation of the mean.

5. van Hooff JP, Boots JM, van Duijnhoven EM, et al. Dosing and management guidelines for tacrolimus in renal transplant patients. *Transplant Proc.* 1999;31:54S–57S.
6. Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin Pharmacokinet.* 2004;43:623–653.
7. van Gelder T. Drug interactions with tacrolimus. *Drug Saf.* 2002;25:707–712.
8. Wallemacq PE, Furlan V, Moller A, et al. Pharmacokinetics of tacrolimus (FK506) in paediatric liver transplant recipients. *Eur J Drug Metab Pharmacokinet.* 1998;23:367–370.
9. Lemahieu W, Maes B, Verbeke K, et al. Cytochrome P450 3A4 and P-glycoprotein activity and assimilation of tacrolimus in transplant patients with persistent diarrhea. *Am J Transplant.* 2005;5:1383–1391.
10. Prescott WA Jr, Callahan BL, Park JM. Tacrolimus toxicity associated with concomitant metoclopramide therapy. *Pharmacotherapy.* 2004;24:532–537.
11. Centers for Disease Control and Prevention. Incidence of end-stage renal disease among persons with diabetes—United States, 1990–2002. *MMWR Morb Mortal Wkly Rep.* 2005;54:1097–1100.
12. Kasiske BL, Snyder JJ, Gilbertson D, et al. Diabetes mellitus after kidney transplantation in the United States. *Am J Transplant.* 2003;3:178–185.
13. Gwilt PR, Nahhas RR, Tracewell WG. The effects of diabetes mellitus on pharmacokinetics and pharmacodynamics in humans. *Clin Pharmacokinet.* 1991;20:477–490.
14. Cashion AK, Holmes SL, Hathaway DK, et al. Gastroparesis following kidney/pancreas transplant. *Clin Transplant.* 2004;18:306–311.
15. van Duijnhoven E, Christiaans M, Undre N, et al. The effect of breakfast on the oral bioavailability of tacrolimus in diabetic and nondiabetic patients before transplantation. *Transplant Proc.* 1998;30:1268–1270.
16. van Duijnhoven E, Christiaans M, Schafer A, et al. Tacrolimus dosing requirements in diabetic and nondiabetic patients calculated from pretransplantation data. *Transplant Proc.* 1998;30:1266–1267.
17. Akhlaghi F, Patel CG, Zuniga XP, et al. Pharmacokinetics of mycophenolic acid and metabolites in diabetic kidney transplant recipients. *Ther Drug Monit.* 2006;28:95–101.
18. Zahir H, Akhlaghi F. Development and validation of a rapid LC-MS/MS technique for simultaneous determination of CsA and TAC in human blood. *The AAPS Journal.* 2004;6:M1017.
19. Center for Drug Evaluation and Research, US Food and Drug Administration. *Bioanalytical Method Validation; Guidance for Industry 2001.* Available at: www.fda.gov/cder/guidance/4252fnl.htm. Accessed January 1, 2007.
20. Faulk JS. Diabetes control and education in transplant care improves outcomes. *Nephrol News Issues.* 2006;20:36, 39, 41.
21. Sheiner LB, Beal SL. Some suggestions for measuring predictive performance. *J Pharmacokinet Biopharm.* 1981;9:503–512.
22. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat Methods Med Res.* 1999;8:135–160.
23. Locatelli F, Pozzoni P, Del Vecchio L. Renal replacement therapy in patients with diabetes and end-stage renal disease. *J Am Soc Nephrol.* 2004;15(Suppl 1):S25–S29.
24. Horowitz M, Edelbroek M, Fraser R, et al. Disordered gastric motor function in diabetes mellitus. Recent insights into prevalence, pathophysiology, clinical relevance, and treatment. *Scand J Gastroenterol.* 1991;26:673–684.
25. Horowitz M, Harding PE, Maddox AF, et al. Gastric and oesophageal emptying in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia.* 1989;32:151–159.
26. Sanaka M, Koike Y, Yamamoto T, et al. A reliable and convenient parameter of the rate of paracetamol absorption to measure gastric emptying rate of liquids. *Int J Clin Pharmacol Ther.* 1997;35:509–513.
27. Kuypers DR, Claes K, Evenepoel P, et al. The rate of gastric emptying determines the timing but not the extent of oral tacrolimus absorption: simultaneous measurement of drug exposure and gastric emptying by carbon-14-octanoic acid breath test in stable renal allograft recipients. *Drug Metab Dispos.* 2004;32:1421–1425.
28. Mekki Q, Lee CC, Carrier S. The effect of food on oral bioavailability of tacrolimus (FK506) in liver transplant patients [Abstract]. *Clin Pharmacol Ther.* 1993;53:229.
29. Zini R, Riant P, Barre J, et al. Disease-induced variations in plasma protein levels. Implications for drug dosage regimens (part I). *Clin Pharmacokinet.* 1990;19:147–159.
30. Zahir H, Nand RA, Brown KF, et al. Validation of methods to study the distribution and protein binding of tacrolimus in human blood. *J Pharmacol Toxicol Methods.* 2001;46:27–35.
31. Jusko WJ, Thomson AW, Fung J, et al. Consensus document: therapeutic monitoring of tacrolimus (FK-506). *Ther Drug Monit.* 1995;17:606–614.
32. Ting LS, Villeneuve E, Ensom MH. Beyond cyclosporine: a systematic review of limited sampling strategies for other immunosuppressants. *Ther Drug Monit.* 2006;28:419–430.
33. Armendariz Y, Pou L, Cantarell C, et al. Evaluation of a limited sampling strategy to estimate area under the curve of tacrolimus in adult renal transplant patients. *Ther Drug Monit.* 2005;27:431–434.
34. Stolk LML, van Duijnhoven EM, Christiaans MHL, et al. Evaluation of prediction of tacrolimus area under the curve by trough concentrations. *Br J Clin Pharmacol.* 2001;53:543P–559P.
35. Wong KM, Shek CC, Chau KF, et al. Abbreviated tacrolimus area-under-the-curve monitoring for renal transplant recipients. *Am J Kidney Dis.* 2000;35:660–666.
36. Kuypers DR, Claes K, Evenepoel P, et al. Time-related clinical determinants of long-term tacrolimus pharmacokinetics in combination therapy with mycophenolic acid and corticosteroids: a prospective study in one hundred de novo renal transplant recipients. *Clin Pharmacokinet.* 2004;43:741–762.