

Time of Drug Administration, CYP3A5 and ABCB1 Genotypes, and Analytical Method Influence Tacrolimus Pharmacokinetics: A Population Pharmacokinetic Study

Flora Tshinanu Musuamba, PharmD,* Michel Mourad, MD, PhD,† Vincent Haufroid, PhD,*
Isabelle Karine Delattre, MSc,* Roger Karel Verbeeck, PhD,‡ and Pierre Wallemacq, PhD*

Abstract: Tacrolimus (TAC) pharmacokinetics are characterized by a very high variability that complicates its therapeutic use. The aims of this study were: 1) to identify and model the effect of demographic, clinical, and genetic factors and time of drug administration on TAC pharmacokinetic variability; and 2) to assess the influence of the analytical method by modeling the TAC blood concentrations measured simultaneously by microparticle enzyme immune assay (MEIA) and liquid chromatography–tandem mass spectroscopy. Data from 19 renal transplant candidates were analyzed. A total of 266 blood samples were analyzed for TAC by both techniques. Linear regression and Bland and Altman analyses were performed to compare TAC blood concentrations obtained with MEIA and liquid chromatography–tandem mass spectroscopy. A population pharmacokinetic analysis was performed. As expected, blood concentrations obtained by MEIA were higher than those obtained by liquid chromatography–tandem mass spectroscopy. A two-compartment model with first-order absorption and elimination best fit TAC blood concentrations. An exponential model was used to describe the interindividual and interoccasion variability and a mixed model was retained for the residual variability. A supplementary proportional term was necessary for the residual error in case of TAC blood concentrations determined by MEIA. The following covariates were retained in the final model: time of drug administration on the absorption rate constant and CYP3A5 and ABCB1 genotypes on the TAC apparent clearance. All parameter estimates had reliable values. The final model was found to be stable and generated parameters with good precision. The validation of the final model by bootstrapping (2000 bootstraps), case deletion diagnostics, crossvalidation, and visual predictive check (1000 simulated subjects) gave satisfactory results. This is the first population pharmacokinetic study confirming the chronopharmacokinetics of TAC and showing an effect of ABCB1 genotype and analytical method on TAC pharmacokinetics. These results may be helpful for TAC dose individualization.

Key Words: tacrolimus, pharmacokinetics, chronopharmacokinetics, therapeutic drug monitoring, immunoassay

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INTRODUCTION

Tacrolimus (TAC) is an immunosuppressive agent produced by *Streptomyces tsukubaensis* and used in combination with mycophenolic acid or corticosteroids for the prevention of acute rejection after solid organ transplantation.¹ It acts primarily through inhibition of calcineurin-mediated T-lymphocyte activation.² This not only results in the reduction of lymphocyte levels, the target effect, but also explains side effects such as neurotoxicity and nephrotoxicity.^{3,4}

When orally administered, TAC has a low bioavailability averaging 20%. Administration with food decreases the rate and extent of TAC absorption with the greatest effect occurring with a high-fat meal. TAC serves as a substrate of gastrointestinal tract p-glycoprotein and is metabolized by cytochrome P450 3A enzymes (CYP3A4 and 5) in the liver and gut lining. At least 15 active and inactive metabolites are produced. Genetic polymorphisms have been identified for the efflux pump and each of these enzymes. Tacrolimus is highly bound to both albumin and α_1 -acid glycoprotein. It also binds to erythrocytes and lymphocytes.³

The pharmacokinetics (PK) of TAC are characterized by a considerable inter- and inpatient variability. In addition, TAC has a rather narrow therapeutic window. As a consequence, dose individualization and TAC therapeutic drug monitoring to determine the actual exposure may improve the efficacy and tolerability of TAC and is currently recommended.^{5–7}

It has been demonstrated in rodents that TAC PK, activity, and toxicity are influenced by the time of drug administration.⁸ As a result of the low number of human studies, the chronopharmacologic activity and toxicity, together with the chronopharmacokinetics of TAC, remain controversial issues.^{9,10}

One of the causes of discrepancies between results published for TAC can be found in the differences in the analytical methods used to quantify TAC in patients' blood specimens. These methods include immunoassays, displaying different profiles of specificity, and chromatographic methods. To date, immunoassays are the most frequently used methods for TAC quantification, whereas liquid chromatography with

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From the Université catholique de Louvain,*Louvain Centre for Toxicology and applied Pharmacology; †Cliniques universitaires St. Luc, Abdominal Surgery Department; and ‡Unité PMNT, Brussels, Belgium.

Correspondence: Pierre Wallemacq, PhD, Cliniques Universitaires St. Luc, 10 Hippocrate Avenue, Brussels 1200, Belgium (e-mail: pierre.wallemacq@uclouvain.be).

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tandem mass–spectrometric detection (LC-MS/MS) is considered the method of reference.⁶

The aims of the present study were: 1) to identify and model the effect of demographic, clinical, and genetic factors and time of drug administration on TAC pharmacokinetic variability by using nonlinear, mixed-effects modeling techniques; and 2) to assess the influence of the analytical method by analyzing the TAC blood concentrations measured in the same patients by microparticle enzyme immunoassay (MEIA) and by LC-MS/MS.

PATIENTS AND METHODS

Patient Characteristics and Study Design

Nineteen adult renal allograft candidates (ie, patients on the waiting list for renal transplantation) in one Belgian university hospital (Cliniques Universitaires Saint Luc, Brussels, Belgium) were included in this study. All patients received oral TAC (0.1 mg/kg body weight) twice daily, ie, at 8:00 AM and 8:00 PM. The morning dose was given after overnight fasting and 1 hour before breakfast and the second dose was administered in the evening 1 hour before dinner. The pharmacogenetic aspects related to the study have been published elsewhere.¹¹ Full PK profiles for TAC during two dosing intervals were determined after the morning and the evening doses. For the determination of the full pharmacokinetic profiles, 2-mL blood samples were collected in EDTA tubes and kept frozen at -20°C until analyzed. Sampling times were as follows: before (0) and at 1, 2, 4, 8, and 12 hours after the morning and evening TAC doses. The patients did not receive any other immunosuppressive drugs, including corticosteroids. Although concomitant nonimmunosuppressive medications were not part of the exclusion criteria, no other medications some patients were taking at the time of the study were known inducers/inhibitors of CYP3A and/or P-glycoprotein.

The protocol was approved by the local ethics committee, and all patients gave written informed consent.

Analytical Method

Microparticle Enzyme Immunoassay

The immunoassay used for all blood specimens was the MEIA performed on the IMx analyzer from Abbott Diagnostics (Wiesbaden, Germany). This method, linear from 1 to 30 ng/mL, was found to be precise on an interday basis: coefficient of variation less than 11% for all quality control samples tested. By MEIA, tacrolimus metabolites: 13-O-demethyl tacrolimus (MI), 31-O-demethyl tacrolimus (MII), 15-O-demethyl tacrolimus (MIII), and 12-hydroxytacrolimus (MIV) are known to display crossreactivity with the antibody of less than 1%, 109%, 90.5%, and 8.8%, respectively.¹² The laboratory successfully participated in the Tacrolimus International Proficiency Testing Scheme (David Holt, Analytical Services International, UK).

Liquid Chromatography–Tandem Mass Spectrometry

A Quattro micro tandem mass-spectrometer (Waters, Manchester, UK) fitted with a Z-Spray ion source was used for tacrolimus analyses after appropriate extraction. Briefly, zinc sulfate solution (40 μL of 0.1 M) was added to 10- μL aliquots of the calibrators and whole blood samples and all samples were mixed briefly. Proteins were precipitated by adding 100 μL methanol that also contained the internal standard (ascomycin, 2 $\mu\text{g/L}$). The tubes were vortex-mixed vigorously for 5 seconds to ensure complete protein precipitation and cellular lysis and then centrifuged for 5 minutes at 10,500 g (Heraeus Labofuge, Hanau, Germany). Twenty microliters of the whole blood extract was injected onto the column maintained at 55°C , which was eluted at a flow rate of 0.6 mL/min with a step gradient of a mixture of solvents A and B (50:50, at time 0 and then switched to 100% solvent B during the period 0.4 to 0.8 minute). Solvent A was used as the “purge solvent” and solvent B was used for the “syringe wash.” Both contained formic acid and ammonium acetate, which are used to promote the formation of ammoniated precursor ions that could easily be fragmented. TAC and ascomycin were monitored by detecting specific product ions resulting from the fragmentation of their ammoniated precursor ions using multiple reaction monitoring acquisition mode. High-purity argon was used as the collision gas. Ionization was achieved in the positive ion mode using the following settings: capillary voltage 1.0 kV; cone voltage 25 V; source block temperature 140°C ; desolvation temperature 350°C at a nitrogen flow of approximately 600 L/h; collision gas pressure 5×10^{-3} mbar; collision energy 18 eV; extractor voltage 3 V; RF lens voltage 0.4 V; exit lens voltage -1 V; entrance lens voltage 1 V; and photomultiplier voltage 650 V. The first quadrupole (MS1) was set to select the ammonium adducts $[\text{M}+\text{NH}_4]^+$ of TAC and ascomycin. The second (MS2), as hexapole, was used as a collision chamber, and the third quadrupole (MS3) was then used to select the characteristic and intense product ions of TAC and ascomycin. The method was linear from 0.5 to 50 ng/mL and was found to be accurate precise on an inter- and intraday basis: bias and coefficient of variation were less than 15% for all quality control samples tested. Limit of detection and limit of quantification were 0.1 and 0.5 ng/mL, respectively.

Assay Comparison

Tacrolimus concentrations measured both by MEIA and LC-MS/MS analysis from adult kidney transplant candidates were used to evaluate the performance of IMx in the clinical setting using LC-MS/MS as a reference. Tacrolimus LC-MS/MS concentrations were plotted against their corresponding MEIA values and against the difference between the two methods as described by Bland and Altman.¹³ In the absence of knowledge of the actual true value, concentrations obtained by the LC-MS/MS method are considered as the best estimate available. Using JMP 6/SAS (Cary, NC) software, a regression equation describing the line of best fit between results of the two assays was calculated and the standard error of this regression slope determined; 95% confidence intervals were also computed on the slope and the intercept of this line.

Agreement between the two methods was assessed by calculating the mean relative prediction error (MRPE) (estimated by the mean difference of the differences between the two methods) and the root mean squared error (RMSE). The RMSE was used to characterize the precision of the assay and the prediction error to estimate the bias on each difference between MEIA and LC-MS/MS. The lower the values for these two parameters, the higher the agreement between the methods. Based on recommended criteria of guidance for analytical method validation, and based on possible therapeutic implications considering the TAC trough concentration therapeutic window (5–15 ng/mL), methods were considered to have significantly different results when values of MRPE and relative RMSE were higher than 20%. Equations 1 and 2 display expressions of estimation of RMSE and MRPE, respectively:

$$RMSE = \frac{1}{N} \sqrt{\sum (C_{IMX} - C_{LC-MS/MS})^2} \quad \text{Eq.1}$$

$$MRPE = \frac{1}{N} \sum \left(\frac{C_{IMX} - C_{LC-MS/MS}}{C_{LC-MS/MS}} \times 100 \right) \quad \text{Eq.2}$$

where C_{IMX} and $C_{LC-MS/MS}$ were TAC concentrations obtained by MEIA and LC-MS/MS, respectively.

Genotyping Analysis

CYP3A5 and ABCB1 genotypes were determined from patients' blood. Two CYP3A5 variant alleles, ie, CYP3A5*3 and CYP3A5*6¹⁴ and three ABCB1 single nucleotide polymorphisms, ie, 1236C > T, 2677G > T/A, and 3435C > T, were determined by restriction fragment length polymorphism analysis as described elsewhere.¹⁵

Population Pharmacokinetics Analysis

Nonlinear mixed effects modeling was performed by using NONMEM Version VI (double precision; Icon Development Solutions, Ellicott City, MD) and PsN-toolkit,¹⁶ a programming library containing a collection of computer-intensive statistical methods for nonlinear mixed effects modeling, and Xpose 4.0,¹⁷ an S-PLUS based population PK/pharmacodynamic model building aid with an interface containing graphic and statistical tools. The first-order conditional estimation approach with interaction between parameters was used throughout the entire modeling process. TAC IMx and LC-MS/MS blood concentrations were modeled and different structural models were tested: one-, two- and three-compartment models with first-order or zero-order absorption and with or without a lag time. Pharmacokinetic parameters of TAC were estimated by NONMEM in terms of first-order absorption rate constant (K_{12}), clearance (CL), volumes of distribution (V) of the various compartments and intercompartmental clearance Q using conventional equations. Because oral bioavailability (F) could not be determined, values for CL, V, and Q correspond to the ratios CL/F, V/F and Q/F.

Interindividual variability in the PK parameters (IIV) and between-occasion variability (morning or evening dose) were modeled using an exponential error model and all

parameters were initially tested. The value of a parameter in the i^{th} individual at the j^{th} occasion (P_{ij}) was a function of the typical value of the parameter (θ) and of the individual deviation initially represented by η_i and K_{ij} representing the interpatient and the between-occasion variability terms for the j^{th} occasion for the i^{th} patient, respectively. The η_s and K_s in the population were supposed to be symmetrically distributed, zero-mean random variables with a variance that is estimated as part of the model estimation from Equation 3:

$$P_{ij} = \theta \times \exp(\eta_i + K_{ij}) \quad \text{Eq.3}$$

η and/or K terms were maintained in the structural model only when they improved the model based on the decrease of the Bayesian information criterion computed as described subsequently.

Additive, proportional, exponential, and mixed error models were tested for the residual error as shown in Equations 4–7:

$$Y = IPRED + \epsilon_{\text{add}} \quad \text{Eq.4}$$

$$Y = IPRED(1 + \epsilon_{\text{prop}}) \quad \text{Eq.5}$$

$$Y = IPRED(\exp(\epsilon_{\text{exp}})) \quad \text{Eq.6}$$

$$Y = IPRED(1 + \epsilon_{\text{prop}}) + \epsilon_{\text{add}} \quad \text{Eq.7}$$

where Y represents the observed concentration; IPRED is the individual predicted concentration; and ϵ_{add} , ϵ_{prop} , and ϵ_{exp} are the additive, the proportional, and the exponential error terms on TAC concentrations, respectively. ϵ_s were supposed to be symmetrically distributed, zero-mean random variables with variance terms that are estimated as part of the population model-fitting process from Equations 4–7.

Model selection only referred to models for which the NONMEM minimization process was successful and was based on the following criteria: the Bayesian information criterion (BIC), the plausibility and the precision of parameter estimates, and graphic analysis. The Bayesian information criterion was computed on the model objective function value (OFV),¹⁸ the number of observations used during the modeling process, and the number of parameters used (NPAR) as follows:

$$BIC = OFV + (\text{LnN} \times \text{NPAR}) \quad \text{Eq.8}$$

The models with the lowest BIC were further evaluated. Precision of parameter estimates, expressed as standard error of estimates, were generated by the covariance option within the NONMEM program. Goodness-of-fit plots, including predictions and individual predictions versus observed concentrations as well as conditional weighted residuals¹⁹ versus predictions, and conditional weighted residuals versus time after dose, were used for diagnostic purposes.

To explain interpatient, interoccasion, and residual variability on PK parameters, relationships were investigated between pharmacokinetic parameters and the following patient covariates: age, sex, race, weight, CYP3A5 and ABCB1 genetic polymorphisms, time of drug intake, analytical method, and total plasma protein concentration. Individual Bayesian estimates of pharmacokinetic parameters

were generated and their correlation with each covariate was evaluated separately using NONMEM. A difference of at least 3.84 of OFV (χ^2 P value ≤ 0.05) from the structural model OFV was considered statistically significant. Covariates that were continuous variables (age, weight, total plasma protein concentration, GOT, GPT, serum creatinine, serum urea) were centered to their median values and tested on the PK parameters in a linear (Eq. 9) or nonlinear (Eq. 10) manner. For example:

$$CL/F = \theta_{CL} + \theta_{WTonCL} \times WT/WT_{med} \quad \text{Eq.9}$$

$$CL/F = \theta_{CL} + \left(\frac{WT}{WT_{med}} \right)^{\theta_{WTonCL}} \quad \text{Eq.10}$$

where θ_{CL} is the population average apparent clearance and θ_{WTonCL} is the fractional change on the apparent clearance resulting from the weight, WT is the patient body weight, and WT_{med} is the median body weight in the data set.

For the categorical covariates, such as sex, race, time of drug intake (occasions 1 and 2 represented daytime and nighttime dose, respectively), CYP3A5 and ABCB1 genotypes, a change in PK parameter, eg, K_{12} , was evaluated by the following type of equation:

$$K_{12} = \begin{cases} \theta_{K12} & \text{in case of morning dose} \\ \theta_{K12} - \theta_{\text{nighttime on } K12} & \text{in case of evening dose} \end{cases} \quad \text{Eq.11}$$

where θ_{K12} is the population average first-order absorption rate constant after the morning dose and $\theta_{\text{nighttime on } K12}$ is the additive change in K_{12} after the evening dose.

or

$$CL/F = \begin{cases} \theta_{CL} & \text{in case of *3/*3 carrier for} \\ & \text{CYP3A5 polymorphism} \\ \theta_{CL} + \theta_{CYPonCL} & \text{in case of *1/*3 or} \\ & \text{*1/*1 carrier for CYP3A5} \\ & \text{polymorphism} \end{cases} \quad \text{Eq.12}$$

where θ_{CL} is the population average apparent clearance for *3/*3 carrier for CYP3A5 polymorphism and θ_{CYP} is the additive change in apparent clearance in case of *1 allele carrier for CYP3A5 polymorphism. Continuous covariates (age, weight, total plasma protein concentration, GOT, GPT, serum creatinine, serum urea) were also categorized and tested as described and/or combined and tested as a unique factor.

A full model was built using NONMEM including all covariates that showed significant influence on PK parameters. A backward process was implemented to build the final model; only covariates which on deletion generated an increase in OFV of more than 11 (χ^2 P value ≤ 0.001) were retained in the final model.

Bootstrapping, case deletion diagnostics followed by crossvalidation and simulations were used to internally validate the final model. Two thousand bootstraps were generated using the PsN toolkit and a confidence interval was built around the median of each parameter. Estimated values of each parameter obtained by the final model were compared with this confidence interval. From the case deletion diagnostics, a Cook score and a covariance ratio were computed for each individual. Patients with both parameter

TABLE 1. Demographic and Biologic Characteristics of the Patient Population: Values are Given as Median (range)

Patient Characteristics (units)	Median [range]
Weight (kg)	67 (50–96)
Age (years)	42 (23–78)
Sex	16 males, 3 females
Tacrolimus dose (mg)	7 (5–10)
Plasma proteins (mg/100 mL)	6.8 (5.1–7.4)
CYP3A5 intron 36986 G > A status	9 CYP 3A5*1
ABCB1 exon 12 1236 C > T status	13 CC
ABCB1 exon 21 2677 G > T/A status	13 GG
ABCB1 exon 26 3435 C > T status	13 CC
Race	14 whites, 5 blacks
Serum creatinine (mg/dL)	5 (1.8–16.4)
Serum urea (mg/dL)	141 (54–195)
Serum GPT (mg/dL)	14 (10–30)
Serum GOT (mg/dL)	13 (9–18)
Total bilirubin (mg/dL)	0.8 (0.6–1.4)

values higher than 1 were considered as influential individuals. From the crossvalidation, RMSE and relative prediction error were computed on the OFV. Values higher than 30% would also suggest the presence of influential individuals. Finally, the predictive performance of the model was evaluated using a visual predictive check. The population PK model was used to simulate 1000 hypothetical patients. The distribution (median and 5th and 95th percentiles) of the simulated concentration-time curves was compared with the

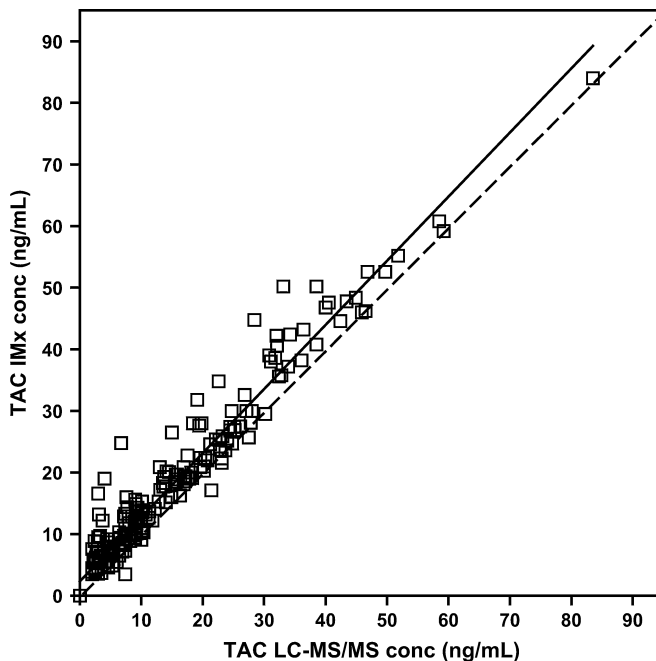


FIGURE 1. Linear regression between tacrolimus concentrations obtained by liquid chromatography–tandem mass spectrometry and IMx. (—), regression line; (---), identity line.

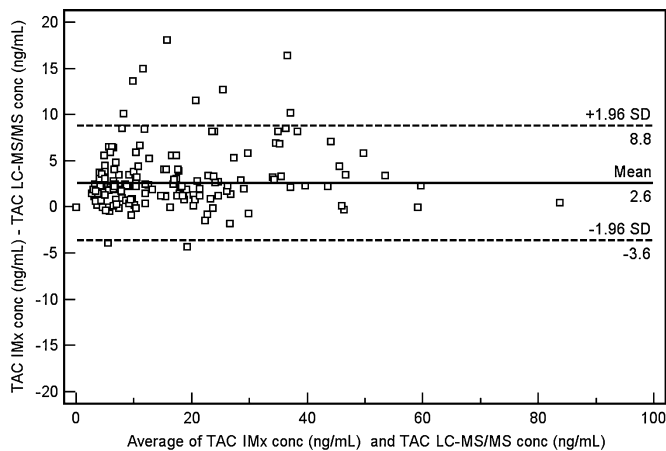


FIGURE 2. Bland and Altman method comparison plot of tacrolimus concentrations obtained by liquid chromatography–tandem mass spectroscopy and IMx. (---), mean difference line; (—), 95% confidence interval line.

observed MEIA and LC-MS/MS TAC values in the original data set.

RESULTS

Clinical characteristics of the patients are summarized in Table 1. Figures 1 and 2 show a linear regression between TAC concentrations obtained by LC-MS/MS and IMx ($r^2 = 0.94$, RMSE = 4, MRPE = 26.4%) and a Bland and Altman method comparison plot, respectively. Good agreement was found between the results obtained by both methods, although IMx values were, as expected, generally higher than LC-MS/MS values. The following prediction expression was found to best correlate IMx and LC-MS/MS concentrations: $TAC_{IMx\ conc} = 1.05 \times TAC_{LC-MS/MS\ conc} + 2.15$. The 95% confidence intervals on the slope and on the intercept were [1.02 to 1.09] and [1.47 to 2.83], respectively. Figure 3 shows individual TAC whole-

blood concentration-time profiles. A large variability was observed.

As far as the basic deterministic model for the population analysis is concerned, a two-compartment model with first-order absorption and elimination best fitted the TAC blood concentrations, irrespective of the assay methodology. The interindividual variability was modeled by an exponential model, and a mixed model was retained to describe the residual error. Nevertheless, supplementary additive and proportional error terms were needed in the case of IMx concentrations. The following PK parameters were estimated: absorption rate constant (K_{12}), apparent volume of the central compartment (V_2/F), apparent volume of the peripheral compartment (V_3/F), apparent intercompartmental clearance (Q/F), and apparent clearance (CL/F) (Table 2). The following covariates showed significant influence on PK parameters: time of drug administration on absorption (K_{12}) and CYP3A5*3 and ABCB1 genotypes on CL/F , all included in the model in an additive manner as shown in Equations 11 and 12. In the final backward elimination step, a significant increase in OFV was observed on elimination of either CYP3A5 or ABCB1 genetic polymorphisms and/or time of drug administration from the model. Therefore, these three covariates were included in the final population pharmacokinetic model. In Table 3, the final model parameters including these covariates are summarized. Good estimation of all the model parameters was obtained (standard error of estimates less than 20% of estimates). Figures 4–6 show diagnostic plots of the performance of the final model. The validation of the final model by bootstrapping and case deletion diagnostics gave satisfactory results. Indeed, all observed parameter values had a CV of 7% to 45% and were included in the 60% to 90% confidence interval computed with the values derived from 2000 bootstraps. Cook’s distance and covariance ratio were lower than 1 for all the study subjects. An RMSE of 12 and an relative prediction error of 30% were found on the objective function. The distribution (5th and 95th percentiles) of the 1000 simulated concentration-time curves and the comparison with the observed IMx and LC-MS/MS TAC

FIGURE 3. Tacrolimus individual pharmacokinetic profiles after administration of 0.1 mg/kg twice daily (N = 19). Concentrations were successively quantified by liquid chromatography–tandem mass spectroscopy (right panel) and IMx (left panel) and visual predictive check (VPC) results on tacrolimus IMx (left panel) and liquid chromatography–tandem mass spectroscopy (right panel) concentrations: solid line (---), median simulated profile line; dashed line (—), limits of 90% prediction intervals on simulated concentrations; open circles (O), tacrolimus observed concentrations.

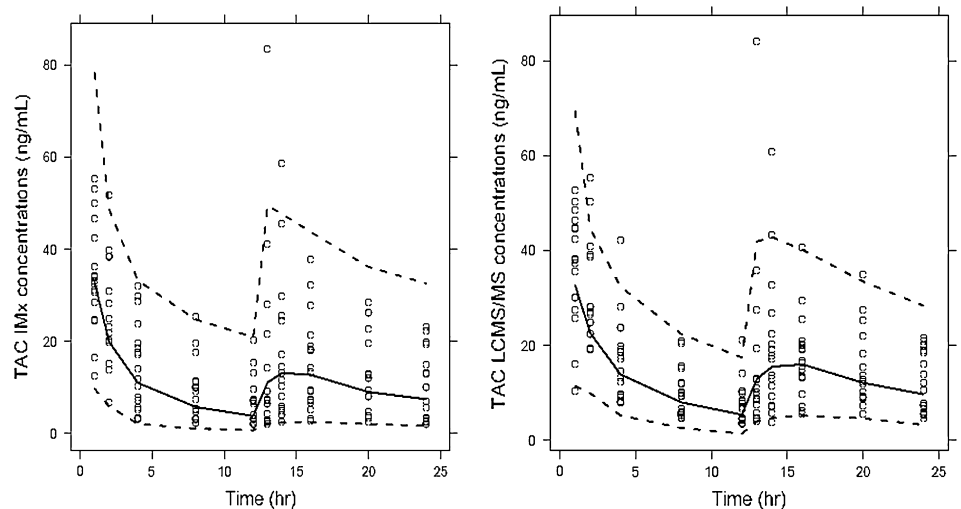


TABLE 2. Population Pharmacokinetic Analysis for the Combined Tacrolimus Blood Concentrations: Structural Model Characteristics (N = 19)

Parameters (units)	Estimates [RSE]	IIV (CV%) [RSE]	IOV (CV%) [RSE]
$\theta_{k_{12}}$ (h^{-1})	0.7 (0.16)	—	187 (2.34)
θ_{V_2} (L)	140 (0.18)	3.0 (1.25)	—
θ_{V_3} (L)	202 (0.23)	14 (0.37)	—
θ_Q (L/h)	29 (0.13)	—	—
θ_{CL} (L/h)	29 (0.20)	30 (0.67)	53 (0.58)
ϵ_{prop}	0.28 (0.20)	NA	—
ϵ_{add} (ng/mL)	0.02 (0.05)	NA	—
$\epsilon_{prop, MEIA}$	0.24 (0.23)	NA	—

θ , population parameter; ϵ_{prop} , coefficient of variation on the proportional term of the residual error; $\epsilon_{prop, MEIA}$, coefficient of variation on the supplementary proportional term of the residual error in case of microparticle enzyme immunoassay concentrations; ϵ_{add} , standard deviation on the additive term of the residual error; IIV, interindividual variability; IOV, interoccasion variability; NA, not applicable; RSE, relative standard error of estimates.

blood concentrations in the original data set are shown in Figure 3. The overlap of the simulated and original distributions indicates the accuracy of the identified final model. This was in agreement with the low rates of false-positives (0.3%) and false-negatives (6%) obtained from visual predictive check.

DISCUSSION

A number of analytical methods, including different immunoassays (enzyme-linked immunosorbent assay, MEIA, enzyme multiplied immunoassay technique, antibody conjugated magnetic immunoassay, chemiluminescent

TABLE 3. Population Pharmacokinetic Analysis for the Combined Tacrolimus Blood Concentrations: Final Model Characteristics (N = 19)

Parameters	Estimates [RSE]	IIV (CV%) [RSE]	IOV (CV%) [RSE]
$\theta_{k_{12}}$ (h^{-1})	2.18 (0.15)	—	—
θ_{V_2} (L)	142 (0.15)	33 (0.45)	—
θ_{V_3} (L)	192 (0.17)	31 (0.43)	—
θ_Q (L/h)	43 (0.14)	—	—
θ_{CL} (L/h)	22 (0.11)	6 (1.08)	40 (0.23)
$\theta_{k_{12, nighttime}}$ (h^{-1})	2.02 (0.16)	NA	—
$\theta_{CYP3A5onCL}$ (L/h)	34 (0.19)	NA	—
$\theta_{ABCB1onCL}$ (L/h)	10 (0.21)	NA	—
ϵ_{prop}	0.29 (0.07)	NA	—
ϵ_{add} (ng/mL)	0.02 (0.12)	NA	—
$\epsilon_{prop, MEIA}$	0.22 (0.23)	NA	—

θ , population parameter; $\theta_{k_{12, nighttime}}$, fractional change in K_{12} after the evening dose; $\theta_{CYP3A5onCL}$, $\theta_{ABCB1onCL}$, effect of the CYP3A5 or of ABCB1 on clearance; ϵ_{prop} , coefficient of variation on the proportional term of the residual error; $\epsilon_{prop, MEIA}$, coefficient of variation on the supplementary proportional term of the residual error in case of microparticle enzyme immunoassay concentrations; ϵ_{add} , standard deviation on the additive term of the residual error; IIV, interindividual variability; IOV, interoccasion variability; NA, not applicable; RSE, relative standard error of estimates.

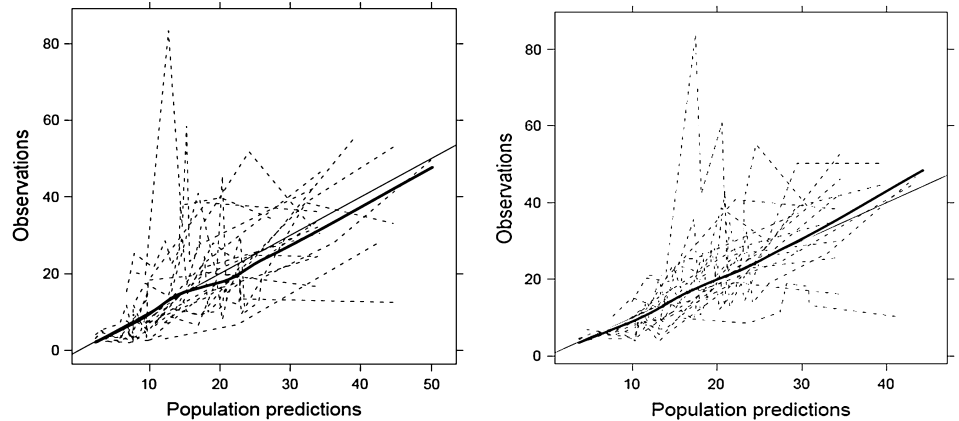
microparticle immunoassay), and liquid chromatographic methods such as LC-MS/MS have been developed to determine tacrolimus concentrations in blood.^{20–22} To date, most PK and PK–pharmacodynamic studies of tacrolimus used immunoassays with MEIA the most frequently used method.⁶ In these immunoassays, the possibility of cross-reactivity between TAC and some of its metabolites with the antibody has to be considered. It has been estimated that up to 30% of the measured TAC blood concentrations could be the result of crossreactivity with TAC metabolites. Indeed, the four major TAC metabolites, ie, 13-O-demethyl tacrolimus, 31-O-demethyl tacrolimus, 15-O-demethyl tacrolimus, and 12-hydroxy tacrolimus, display the following percentages of crossreactivity: 0%, 109%, 90.5%, and 8.8% as compared with tacrolimus.²² In addition, the immunosuppressive activity of these metabolites, evaluated by the IC_{50} from the mixed lymphocyte reaction, does not correlate with their degree of crossreactivity with the antibodies used in the various immunoassays, causing some difficulties in the final interpretation of the results.²³ A better understanding of the influence of the analytical method used for TAC dose individualization based either on trough concentrations or on AUC_{12} is of particular importance.⁶

LC-MS/MS is a specific assay for the parent drug, thus avoiding the problem of metabolite interference. An increasing number of transplant centers are now using LC-MS/MS analysis for therapeutic drug monitoring of tacrolimus. The opportunity for simultaneous measurement of coadministered drugs such as sirolimus, everolimus, and mycophenolic acid makes this method both clinically and economically interesting.

One of the objectives of this study was to compare PK parameters of TAC in adult kidney transplant candidates, and therefore dose adjustment, based on TAC blood concentrations determined by MEIA and LC-MS/MS. A good correlation was found between both analytical methods ($r^2 = 0.94$), but the MEIA assay showed a bias (MRPE) of +26% with a relative imprecision (rRMSE) of 25% in comparison to LC-MS/MS. The results obtained in this study show that the analytical method has a direct impact on PK parameters as computed with the noncompartmental approach. This discrepancy was modeled using the population PK approach through a supplementary residual error model based on the analytical method. The results of this study show that the therapeutic window of TAC trough concentrations in blood, which is generally considered to be 5 to 15 ng/mL,⁴ based on immunoassays (mostly MEIA) determinations, depends on the analytical method used and should be decreased when TAC blood concentrations are quantified by LC-MS/MS. Our findings are in agreement with those reported by Staatz et al,²³ who compared an enzyme-linked immunosorbent assay method with LC-MS/MS and indicate that the target trough concentrations and target area under the curve (AUC) should be adjusted according to the analytical method used to quantify TAC in the patient's blood samples. This is important, because dose adjustment is based on a selected target trough concentration or AUC_{12} .

This is the first population PK report of TAC that analyzes the influence of the analytical method (MEIA versus

FIGURE 4. Population-predicted versus observed tacrolimus IMx (left panel) and liquid chromatography–tandem mass spectroscopy (right panel) concentrations (ng/mL) in the final population pharmacokinetic model: fine line (---), identity line; bold line (—), regression line; dashed line (---), patient concentrations joining line.



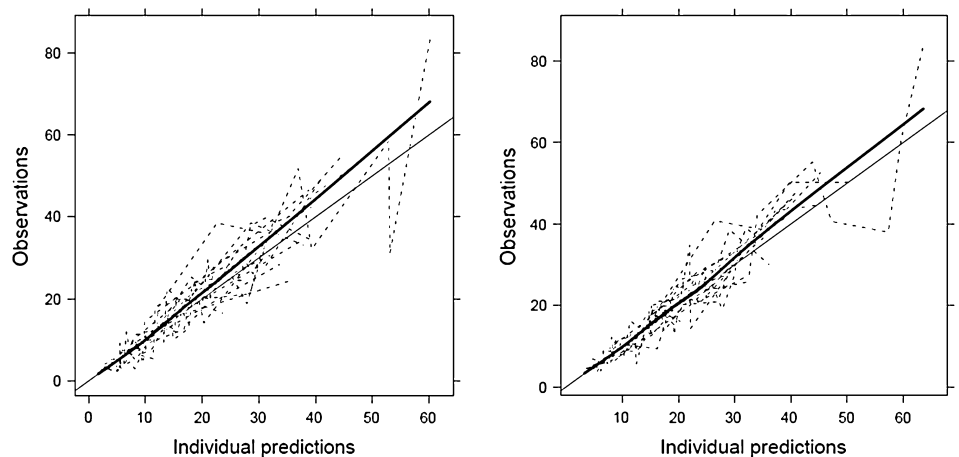
LC-MS/MS) in addition to the impact of daytime versus nighttime dosing in the same patients. A two-compartment model with first-order absorption and elimination rates best described the data. A population PK model for TAC was developed by using nonlinear mixed effects modeling and validated by goodness-of-fit plots, precision of estimates, bootstrapping, and simulation-based diagnostics.

During the modeling process, the minimization was successful with the one-compartment model, and the parameter estimates had good precision and credible values, but the AIC criterion was in favor of the two-compartment model. In addition, structural model goodness-of-fit plots showed better performance for the two-compartment model. Several population PK models have been described for TAC in the de novo and stable transplant patients.^{24–33} In most cases, a biexponential elimination model with first-order absorption and sometimes an absorption lag time was selected to describe the data. Based on therapeutic drug monitoring data, a one-compartment model is most frequently used, probably as a result of the lack of data during the absorption and distribution phases.³⁴

Inclusion of the following three covariates in the population PK model based on TAC blood concentrations measured by LC-MS/MS significantly reduced the interpatient

variability of certain PK parameters: time of drug administration on K_{12} (ie, first-order absorption rate) and CYP3A5*3 and ABCB1 genotypes on CL/F. However, body weight did not influence either the clearance or the central distribution volume. In population PK studies of TAC reported previously the ABCB1 genetic polymorphism was not identified as a significant covariate, probably as a result of the fact that results were based on TAC blood concentrations measured by immunoassays. Indeed, during our analysis, in the population PK model based solely on TAC blood concentrations determined by MEIA, ABCB1 genotype was not a significant covariate. The results of the present study with LC-MS/MS suggest that the combination of ABCB1 and CYP3A5*3 genetic polymorphisms has a relatively strong influence on the interpatient variability of TAC clearance. Indeed, the inclusion of these two covariates explained 13% of the interoccasion and 24% of the interindividual variability of TAC clearance, and both genotypes should, ideally, be taken into account for dose individualization. In a particular patient, the apparent clearance (CL/F) appears to be threefold higher if the patient is a *1/*1 or *1/*3 carrier for the CYP 3A5*3 polymorphism and a CC-GG-CC carrier for the ABCB1 exons 12-21-26 as compared with the case of a patient who is a *3/*3 for CYP 3A5*3 and a TT-TT-TT or CT-GT-CT for the ABCB1 exons

FIGURE 5. Bayesian predicted versus observed tacrolimus IMx (left panel) and liquid chromatography–tandem mass spectroscopy (right panel) concentrations (ng/mL) in the final population pharmacokinetic model: fine line (---), identity line; bold line (—), regression line; dashed line (---), patient concentrations joining line.



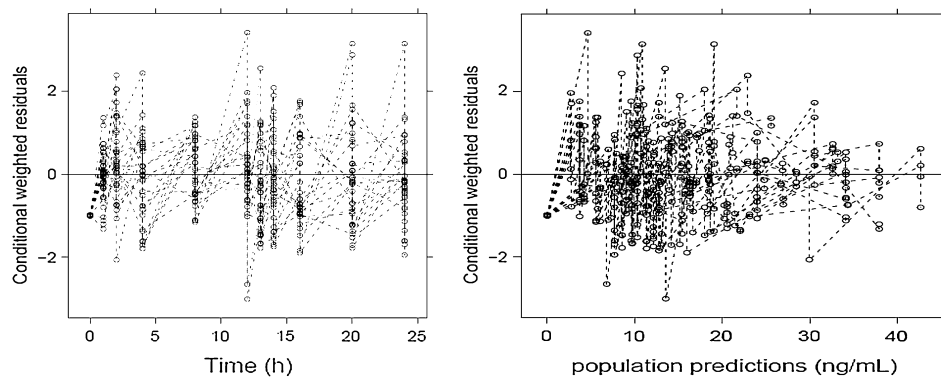


FIGURE 6. Tacrolimus conditional weighted residuals versus predicted concentrations (left panel) and versus time (right panel) in the final population pharmacokinetic model: dashed line (---), patient residuals joining line.

12-21-26. A threefold difference in CL/F means that the maintenance dose to reach the target average concentration is also threefold different.

In this study, time of drug administration was found to influence only the absorption rate constant, but in a very significant way. The average absorption rate constant was 2.18 h^{-1} after the morning dose and 0.16 h^{-1} after the evening dose irrespective of the analytical method. This is in agreement with some, but not all, of the previously published studies showing very different profiles during daytime and nighttime, but with very similar night and day AUC₁₂ values in the same individual. However, the results of some other studies did not show any significant difference between morning and evening TAC PK parameters, concluding that there is no evidence of TAC chronopharmacokinetics. According to our data, because neither the trough concentration nor the AUC are influenced by the time of drug intake, it can be concluded that the circadian variation in TAC absorption rate will not modify patient outcome.

REFERENCES

- Kino T, Hatanaka H, Miyata S, et al. FK506, a novel immunosuppressant isolated from a Streptomyces II. Immunosuppressive effect of FK-506 in vitro. *J Antibiot*. 1987;40:1256–1265.
- Venkataraman R, Swaminathan A, Prasad T, et al. Clinical pharmacokinetics of tacrolimus. *Clin Pharmacokinet*. 1995;29:404–430.
- Scott LJ, McKeage K, Keam SJ, et al. Tacrolimus: a further update of its use in the management of organ transplantation. *Drugs*. 2003;63:1247–1297.
- Staatz CE, Tett SE. Clinical pharmacokinetics and pharmacodynamics of tacrolimus in solid organ transplantation. *Clin Pharmacokinet*. 2004;43:623–653.
- Aweka FT. Comparative pharmacokinetics of orally (PO) and intravenously administered tacrolimus (FK506) in pre- and post-kidney transplant recipients [Abstract]. *Clin Pharmacol Ther*. 1993;53:151.
- Wallemacq P, Armstrong VW, Brunet M, et al. Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference. *Ther Drug Monit*. 2009;31:139–152.
- Mekki QA. Pharmacokinetics of FK506 in kidney transplant patients [Abstract]. *Clin Pharmacol Ther*. 1993;53:151.
- Fujimura A, Shiga T, Ohashi K, et al. Chronopharmacokinetic study of a new immunosuppressive agent, FK 506, in mice. *Jpn J Pharmacol*. 1993;61:137–139.
- Satoh S, Kagaya H, Saito M, et al. Lack of tacrolimus circadian pharmacokinetics and CYP3A5 pharmacogenetics in the early and maintenance stages in Japanese renal transplant recipients. *Br J Clin Pharmacol*. 2008;66:207–214.
- Baraldo M, Furlanut M. Chronopharmacokinetics of ciclosporin and tacrolimus. *Clin Pharmacokinet*. 2006;45:775–788.
- Haufrond V, Wallemacq P, VanKerckhove V, et al. CYP3A5 and ABCB1 polymorphisms and tacrolimus pharmacokinetics in renal transplant candidates: guidelines from an experimental study. *Am J Transplant*. 2006;6:2706–2713.
- Tamura K, Fujimura T, Iwasaki K, et al. Interaction of tacrolimus(FK506) and its metabolites with FKBP and calcineurin. *Biochem Biophys Res Commun*. 1994;202:437–443.
- Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet*. 1986;1:307–310.
- Homepage of the human cytochrome P450 (CYP) alleles nomenclature Committee. Available at: www.imm.ki.se/CYPalleles. Accessed May 2009.
- Haufrond V, Mourad M, Van Kerckhove V, et al. The effect of CYP3A5 and MDR1 (ABCB1) polymorphisms on cyclosporine and tacrolimus dose requirements and trough blood levels in stable renal transplant patients. *Pharmacogenet Genomics*. 2004;14:147–154.
- Lindbom L, Pihlgren P, Jonsson EN. PsN-Toolkit—a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. *Comput Methods Programs Biomed*. 2005;79:241–257.
- Jonsson EN, Karlsson MO. Xpose—an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. *Comput Methods Programs Biomed*. 1999;58:51–64.
- Bertrand J, Comets E, Mentré F. Comparison of model-based tests and selection strategies to detect genetic polymorphisms influencing pharmacokinetic parameters. *J Biopharm Stat*. 2008;18:1084–1102.
- Hooker AC, Staatz CE, Karlsson MO. Conditional weighted residuals (CWRES): a model diagnostic for the FOCE method. *Pharm Res*. 2007;24:2187–2197.
- Zhang Q, Simpson J, Aboleneen HI. A specific method for the measurement of tacrolimus in human whole blood by liquid chromatography/tandem mass spectrometry. *Ther Drug Monit*. 1997;19:470–476.
- Taylor PJ, Jones A, Balderson GA, et al. Sensitive, specific quantitative analysis of tacrolimus (FK506) in blood by liquid chromatography–electrospray tandem mass spectrometry. *Clin Chem*. 1996;42:279–285.
- Alak AM. Measurement of tacrolimus (FK506) and its metabolites: a review of assay development and application in therapeutic drug monitoring and pharmacokinetic studies. *Ther Drug Monit*. 1997;19:338–351.
- Iwazaki K, Shiraga T, Matsuda H, et al. Further metabolism of FK506 (tacrolimus). Identification and biological activities of the metabolites oxidized at multiples sites of FK506. *Drug Metab Dispos*. 1995;23:28–34.
- Staatz CE, Taylor PJ, Tett SE. Comparison of an ELISA and an LC/MS/MS method for measuring tacrolimus concentrations and making dosage decisions in transplant recipients. *Ther Drug Monit*. 2002;24:607–615.
- Staatz CE, Willis C, Taylor PJ, et al. Population pharmacokinetics of tacrolimus in adult kidney transplant recipients. *Clin Pharmacol Ther*. 2002;72:660–669.
- Antignac M, Hulot JS, Boleslawski E, et al. Population pharmacokinetics of tacrolimus in full liver transplant patients: modelling of the post-operative clearance. *Eur J Clin Pharmacol*. 2005;61:409–416.
- Staatz CE, Willis C, Taylor PJ, et al. Toward better outcomes with tacrolimus therapy: population pharmacokinetics and individualized dosage prediction in adult liver transplantation. *Liver Transpl*. 2003;9:130–137.
- Garcia Sanchez MJ, Manzanares C, Santos-Buelga D, et al. Covariate effects on the apparent clearance of tacrolimus in paediatric liver transplant patients undergoing conversion therapy. *Clin Pharmacokinet*. 2001;40:63–67.

29. Fukudo M, Yano I, Fukatsu S, et al. Forecasting of blood tacrolimus concentrations based on the Bayesian method in adult patients receiving living-donor liver transplantation. *Clin Pharmacokinet.* 2003;42:1161–1178.
30. Willis C, Staatz CE, Tett SE. Bayesian forecasting and prediction of tacrolimus concentrations in pediatric liver and adult renal transplant recipients. *Ther Drug Monit.* 2003;25:158–166.
31. 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.
32. Fukatsu S, Yano I, Igarashi T, et al. Population pharmacokinetics of tacrolimus in adult recipients receiving living-donor liver transplantation. *Eur J Clin Pharmacol.* 2001;57:479–484.
33. Sam WJ, Tham LS, Holmes MJ, et al. Population pharmacokinetics of tacrolimus in whole blood and plasma in Asian liver transplant patients. *Clin Pharmacokinet.* 2006;45:59–75.
34. Sam WJ, Aw M, Quak SH, et al. Population pharmacokinetics of tacrolimus in Asian paediatric liver transplant patients. *Br J Clin Pharmacol.* 2000;50:531–541.
35. Antignac M, Barrou B, Farinotti R, et al. Population pharmacokinetics and bioavailability of tacrolimus in kidney transplant patients. *Br J Clin Pharmacol.* 2007;64:750–757.