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Population Pharmacokinetic Analysis of Tacrolimus in Adult Mexican Patients with Renal Transplant

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ABSTRACT

The aim of this study was to estimate the population pharmacokinetic parameters of Tacrolimus in Mexican adults undergoing kidney transplantation and to identify the clinical factors affecting Tacrolimus pharmacokinetics. Using Non-linear mixed effects modeling (NONMEM) with First-order absorption and elimination, a total of 592 retrospective-prospective drug monitoring data points were collected from 36 patients with renal transplant receiving Tacrolimus (Prograf® twice daily during the first 6 post-transplantation months and a generic product thereafter. The absorption rate constant was fixed at 4.5 h^{-1}, and the following population pharmacokinetic estimates were obtained: Tacrolimus clearance (CL/F), 22.5 L/h, and apparent volume of distribution (V/F), 812 L. Interindividual variability was 52.9 and 82.1% for CL/F and V/F, respectively. The covariates that significantly affect Tacrolimus pharmacokinetics parameters were concomitantly administered calcium channel blocker drug and hematocrit level. The population pharmacokinetic analysis identified important sources of variability in Tacrolimus pharmacokinetics. The model will help to calculate Tacrolimus dose requirements in Mexican renal transplant recipients according to specific clinical factors affecting Tacrolimus clearance and volume of distribution and it will also be useful for therapeutic drug monitoring.

Key words: Tacrolimus pharmacokinetics, Mexican, renal transplant.

INTRODUCTION

Tacrolimus is a calcineurin inhibitor widely used as part of the immunosuppressive regimen in solid organ transplantation. It is characterized by a narrow therapeutic index and large intra- and interindividual pharmacokinetics variability. Tacrolimus oral bioavailability and elimination half-life range from 5-93% (mean, 25%) and from 3.5-50 h, respectively. In whole blood, tacrolimus is mainly bound to 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.[1,2] The drug is extensively metabolized in the intestinal mucosa and in the liver mainly by the Cytochrome P450 3A4 (CYP3A4) enzyme system and its apparent clearance is about 20-30 L/h. [3,4]

Tacrolimus has a narrow therapeutic index, with a target trough concentration in adult kidney transplant recipients ranging from 5-20 ng/mL.[3] As a result, clinicians are often faced with sub- or supra-therapeutic tacrolimus levels, which could result in therapeutic inefficacy or toxicity. Therapeutic drug monitoring (TDM) to achieve target blood concentrations has been useful to improve the efficacy of the treatment and to reduce the toxicity of tacrolimus; however, rejection and toxicity episodes continue to be encountered. In clinical practice, it may take 2 or more weeks to establish an adequate maintenance dose, during which time organ rejection or tacrolimus toxicity may occur due to fluctuating levels.[5] Consequently, it is necessary to achieve the maintenance dose as soon as possible so that the therapeutic efficacy of tacrolimus can be improved and the adverse side effects reduced.[6]

Population pharmacokinetic studies can be used to identify the influence of multiple factors (covariates) on drug pharmacokinetics. Furthermore, population pharmacokinetic models can be employed as a priori information for Bayesian forecasting of individual pharmacokinetic parameters and exposure indices, permitting individual dose adjustment. It is well-established that tacrolimus pharmacokinetics differ across ethnic groups; studies of different populations in adult kidney transplant recipients show different pharmacokinetic profiles in Caucasian males compared with African-Americans and Asian.[7,8] Only a few population pharmacokinetic studies with other drugs have been carried out in Mexico.[9-13] To date, no information on tacrolimus population pharmacokinetics in Mexican adult kidney transplant recipients has been published; thus, this study will provide the information necessary for the tacrolimus individualization regimen based on the pharmacokinetic parameters representative of the population studied.

The aim of this study was to develop a population pharmacokinetic model of tacrolimus including the influence of clinical factors, concomitant medications, and the type of formulation administered on the pharmacokinetic parameters based on routine drug monitoring data in adult Mexican renal transplant recipients.

MATERIALS AND METHODS

Patients and clinical data collection

Adult kidney transplant recipients treated with tacrolimus at the National Institute of Cardiology Ignacio Chávez in Mexico City between 2009 and 2010 were included in this study. The whole population was randomly allocated to the index (model-building) dataset (n = 36) or to the validation dataset (n = 15). Approval for the study was obtained from the Institute's Ethics Committee. These datasets included tacrolimus dose, dosing time, and trough blood concentrations (n=592). Patients' characteristics were collected retrospectively (n=21) and prospectively (n=30) from electronic and patient medical records and included age, sex, height, Body weight (BW), Post-transplantation days (PTD), biological data, hematocrit, hemoglobin, erythrocytes, serum creatinine, and concurrent medication. All patients were orally administered with tacrolimus (Prograf®, Janssen-Cilag. Mexico, D.F.) twice daily during the first 6 post-transplantation months and a generic product thereafter. All trough concentrations were obtained as part of clinical care at the hospital. To ensure the steady-state of tacrolimus levels, only trough concentrations measured after day 2 post-transplantation were used in this analysis. Patients received oral tacrolimus therapy as part of a triple immunosuppressive regimen, which also included

Mycophenolate mofetil and Prednisone. Tacrolimus therapy was generally initiated at a dosage of 0.13 mg/kg twice daily. Subsequent doses were adjusted empirically on the basis of clinical evidence of efficacy and toxicity and in order to maintain tacrolimus trough blood concentrations between 10 and 15 ng/mL during the first 3 months after transplantation, and between 5 and 8 ng/mL thereafter.

Drug analysis

Concentrations of tacrolimus in whole blood were assessed using Chemiluminescent microparticle immunoassay performed by the ARCHITECT System Tacrolimus Assay (Abbott Laboratories, Abbott Park, IL, USA). The low-end precision showed coefficients of variation of 4.9% at 3.0 ng/mL; 4.2% at 8.5 ng/mL; and 4.0% at 15.7 ng/mL. Lower limit of detection was 0.3 ng/mL, and this was linear between 2 and 30 ng/mL, with a correlation coefficient ≥0.90.

Population pharmacokinetic model analysis

Pharmacokinetic analysis was carried out using the nonlinear mixed effects modeling program NONMEM (version VII, level 2.0; ICON Development Solutions, Ellicott City, Maryland, USA), integrated with PDx-POP (version 5, GloboMax LLC, Hanover, MD). The First-order conditional estimation (FOCE) method with subroutines ADVAN 2 TRANS 2 was utilized to estimate pharmacokinetic parameters and their variability.

Structural model

The model was established using the forward inclusion-backward elimination method.[14]The population pharmacokinetic analysis was conducted without any covariates in the basic model. Bioavailability (F) could not be determined because tacrolimus was orally administered and only the troughs levels were measured; therefore, the pharmacokinetic values of clearance and distribution volume corresponded to CL/F (apparent clearance) and V/F (apparent volume of distribution) ratios. Because no data from the absorption phase were available, the absorption rate constant (Ka) was fixed at 4.5 h^{-1}.[4] Initial estimates for other parameters were obtained from the literature.[15,16]

Random effect model

Interindividual variability models of Tacrolimus pharmacokinetic parameters were evaluated using additive, proportional, and exponential models. The residual error model was also tested using an additive, proportional, and combined (proportion plus additive) model.

Covariate model

Scatter plots of CL/F and V/F against each covariate aided in identifying trends and regression patterns. Each covariate was screened in turn by incorporating it into the baseline model to develop the intermediate and full models and by observing the decrease of the Objective function value (OFV). The minimal OFV obtained in this step was employed as a standard for assessing the impact of inclusion and exclusion of different covariates on subsequent models. The covariates that were considered were the following: demographic characteristics (age, BW, and sex); laboratory clinical test data (hematocrit, erythrocyte count, and serum creatinine level); PTD; the formulation administered (innovator product Prograf® or a generic product), and drug interaction with Prednisone and antihypertensive drugs. A stepwise backward elimination procedure was carried out. Each covariate was removed independently from the intermediate model to confirm its relevance. Selection among models was based on minimal value of the NONMEM OFV value [–2 log (likelihood)], goodness-of-fit, and precision of parameter estimates. A covariate was selected in the final population pharmacokinetic model when its addition resulted in a reduction in the OFV of at least 10.8 in the OFV (p<0.05; 1 degree of freedom). There were also many indicators for improvement of fit due to the addition of a parameter to the model as follows: decrease in standard error (SE) of parameter estimates; reduction in inter- and intrapatient variability; correlation between observed and predicted concentrations, and reduction in weighted residuals (WRES). Furthermore, inspection of population predictions (PRED) and individual predictions (IPRED) *vs* observed concentrations (DV) and of WRES *vs* predicted concentrations of tacrolimus allowed assessment of improvement of fit from baseline to final model.[17]

Model evaluation

The final population pharmacokinetic model was quantitatively evaluated by the external validation method. Another 15 patients were included in the validation group. The observed concentrations (n = 199) were compared with the corresponding predictions by NONMEM based on the final model. Bias (Mean prediction error, MPE) and precision (Root mean square prediction error, RMSE) of Population prediction (PRED) and Individual prediction (IPRED) were used to assess the predictive performance. WRES were plotted against predicted concentrations to evaluate deviations among model-predicted and observed concentrations.[18]

RESULTS

Patient Demographics

Demographic characteristics of the patient population studied are presented in Table1. Data were collected from 51 adult kidney recipients (31 males, 20 females). About 15.6 ± 6.4 blood samples (range, 3-32 blood samples) per patient for tacrolimus trough concentration determination were taken from the whole population. Mean daily dose

Table 1. Demographic and Biochemical Data of Study Population

Variables	Population Study (n= 36)		Validation population (n= 15)	
	Mean ± SD	Range	Mean ± SD	Range
Sex (% Male)	67	-	47	-
Age (years)	30.2 ± 9.2	17-52	27.9 ± 5.8	21-46
Weight (Kg)	63.6 ± 13.8	39.2-103	62.4 ± 11.1	45.5-88
Observations per patient	16.5 ± 6.32	6-32	13.3 ± 6.5	3-24
Post-transplantation time (days)	-	2-574	-	2-553
Tacrolimus blood concentration (ng/mL)	7.3 ± 5.7	0.9 - 30	6.7 ± 4.9	2-24.7
Dose of tacrolimus (mg/day)	6.63 ± 3.14	1-14	6.56 ± 3.03	2-14
Dose of tacrolimus (mg/Kg/day)	0.109 ±0.057	0.014-0.25	0.106 ± 0.046	0.022-0.24
Hematocrit (%)	38.1 ± 8.9	17-64.3	38.0 ± 7.9	13.6-64.3
Erythrocytes (10^6/μL)	4.4 ± 1.2	1.3-10.4	4.1 ± 1.0	1.55-7.36
Creatinine (mg/dL)	1.61 ± 1.65	0.57-16.5	1.51 ± 1.18	0.49-14.9

Table 2. Final population pharmacokinetic parameter estimates of tacrolimus

Parameters	Meaning	Estimate	SE(%)	95% IC
θ_1	$C\Lambda/\Phi$	22.5	1.7	21.7-23.3
θ_2	ξ/Φ	812.7	6.06	716.1-909.2
θ_3	$K\alpha$	4.5 (Fix)	-	-
θ_4	Factor for CCB	7.6	1.31	7.5-7.7
θ_5	Factor for HEM	10.2	0.9	9.4-10.9
Parameter	Variability	Estimate	CV(%)	
$\omega_{X\Lambda/\Phi}$	Interindividual Variability on CL/F	0.280	52.9	
$\omega_{\xi/\Phi}$	Interindividual Variability on V/F	0.808	82.1	
ρ	Residual Variability (SD)	15.2	3.97	

CL/F, clearance (L/h); Vd/F, volume of distribution (L);%RSE, per cent relative standard error; CI, confidence interval estimate; CCB, calcium channel blocker at time of through measurement; HEM, hematocrit number; CV, coefficient of variation (%); SD, standard deviation.

and trough blood concentration of tacrolimus varied widely with PTD, with a mean of 0.109 ± 0.057 mg/kg/day. The wide range (0.014-0.25 mg/kg/day) confirmed large interindividual variations even with the utilization of TDM. Data showed large interindividual variations over time after transplantation. Figure 1 shows the trough blood concentrations of tacrolimus during PTD. Thirty nine percent of tacrolimus trough concentrations fell within the range of 10-15 ng/mL in the first 3 months after transplantation and between 5 and 8 ng/mL thereafter; 44% of the concentrations were below the desired range and 17% were above the latter. Among the patients, 52% required antihypertensive treatment. Coadministered antihypertensive drugs included calcium channel blockers (CCB) and angiotensin-converting enzyme inhibitors.

Structural Model

A total of 592 trough whole blood concentrations were available for population modeling. The model was parameterized in terms of Ka, CL/F, and V/F. Intersubject variability was described by the exponential error model.

Covariate Model

In the model-building phase, seven covariates, including PTD, serum creatinine, hematocrit, erythrocytes, tacrolimus product formulation, Prednisone dose, and coadministration of antihypertensive drugs were studied. In the models incorporating single covariates, neither demographic parameters (including the patient's characteristics, age, weight, and sex) nor clinical parameters, such as serum creatinine and number of erythrocytes, were detected as significant covariates. The impact of

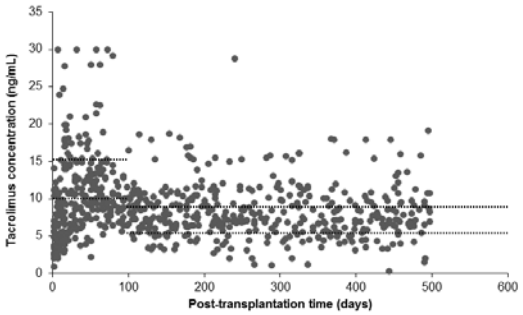

Fig. 1. Tacrolimus trough concentrationsduring the firsts PTD for the whole population. The dotted line represents the established therapeutic ranges

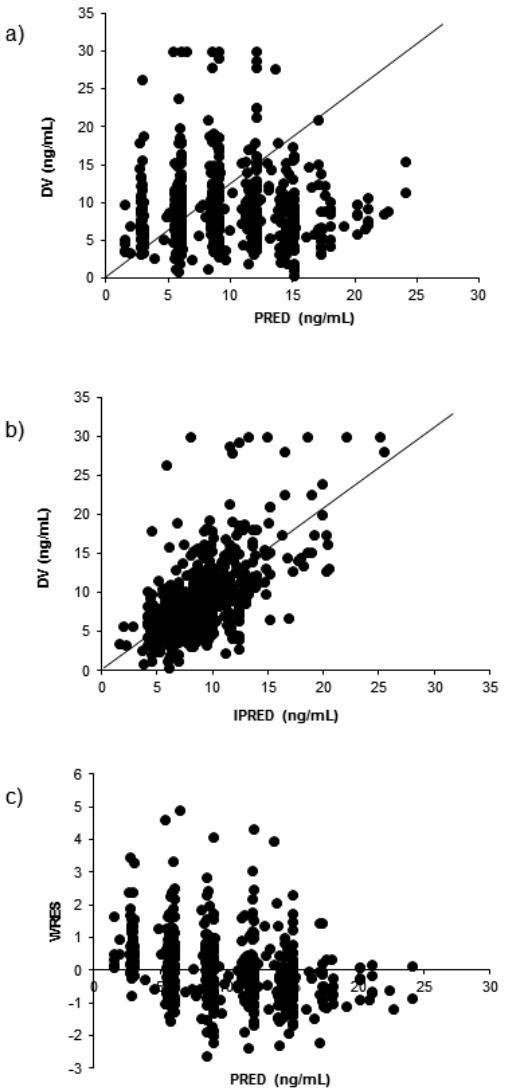

Fig. 2. Diagnostic plots for goodness of fit of the final model (n=36). Upper panel (a) predicted (PRED) *vs* observed concentrations of tacrolimus (DV). Middle panel (b) population predicted (IPRED) *vs* observed concentrations of tacrolimus (DV). Lower panel (c) weighted residual (WRES) versus population predicted concentrations of tacrolimus

PTD was evaluated as a continuous covariate; however, in our analysis the inclusion of this factor exerted no influence on the pharmacokinetic parameters. Although inclusion of tacrolimus product formulation was significant during development of the regression models, in the back elimination step, interindividual variability in CL/F was increased. Consequently, this covariate was removed from the model.

In a (forward) modeling building step, inclusion of antihypertensive drugs and Prednisone produced a decrease in the OFV value by 3.84 or more (p<0.001) when tested against the baseline model. In the (backward) elimination step, only antihypertensive administration was retained. Among the analyzed covariates, erythrocyte fraction was identified as the sole covariate influencing apparent V/F. An exponential error model was selected to describe interindividual variability. An additive model provided best results for residual variability. Table 2 reports the value for each pharmacokinetic parameter determined for the final model.

Model Validation

The robustness of the derived pharmacokinetic parameters was evaluated in an independent validation group (n = 15). Fixed effects were estimated with precision and the 95% Confidence interval (95% CI) did not include the value of zero. Bias (MPE) and precision (RMSE) for the pharmacokinetic baseline model were 0.14 and 3.84 ng/mL and 0.08 and 2.98 ng/mL for the final model, respectively (Table 3). The scatter plot of WRES vs. PRED (Figure 2c) showed that WRES were randomly distributed and fell mostly within ±2 units of the null ordinate.

DISCUSSION

In the present study, we investigated the population pharmacokinetics of tacrolimus in Mexican renal transplant patients and identified the factors affecting its pharmacokinetics. A one-compartment open model with first-order absorption and elimination was optimal for modeling the data; these results agree with those of previously published population analysis studies performed in adults.[15,16,19] The dosage required to reach the target range of tacrolimus trough concentrations when calculated by individual BW showed large interindividual variations during the initial post-transplantation months. It has been suggested that there are several limitations in a weight-based dosage regimen for achieving the target therapeutic tacrolimus concentration during early stages after transplantation. This limitation was also observed in our study population in which, despite dosing based on TDM, the majority of patients reached higher levels than the target trough concentration range during the entire study period (Figure 1). Within this context, identification of predictive parameters for optimal tacrolimus dosage is of major clinical interest for facilitating individualized dosing of this drug. Tacrolimus pharmacokinetic parameters, in conjunction with common clinical covariates, were estimated to build a population model in order to predict individual tacrolimus CL/F and V/F, which may be used in the clinical setting. This evaluation yielded reasonable estimates of the baseline pharmacokinetic parameters of tacrolimus. With the final model, we found a Tacrolimus population mean estimate for CL/F of 22.5 L/h (range, 21.7-23.3 L/h). This value was near those reported in other studies that performed population pharmacokinetic analyses on renal

Table 3. Predictive performance of final model developed from the model building data set (n = 36) and independent data set (n = 15)

Error	Basic model*	Final model*
MPE (bias)	0.14 (-0.16-0.45)	0.08 (-0.2-0.37)
RMSE (precision)	3.84 (0.63-7.04)	2.98 (1.79-4.16)

*95% Confidence interval

transplant recipients.[15,20-24]Part of the interindividual variability in CL/F of tacrolimus was explained by the use of antihypertensive agents by the patients. Antihypertensive medications are commonly used to treat hypertension in patients with a transplant. In our study, 75% of patients receiving antihypertensive drugs were receiving CCB. Pharmacokinetic interactions between tacrolimus and CCB has been reported earlier in renal transplant recipients.[21,24,25]

CCB drugs are CYP3A4 and P-glycoprotein inhibitors[26]. Because CYP3A is responsible for >90% of the metabolic elimination of tacrolimus, inhibition or induction of CYP3A will lead to a clinically significant pharmacokinetic drug interaction.[26,27] The model developed in this study established that administration of CCB agents caused a decrease of 8.5% in the value of CL/F with respect to the estimated value in the baseline model. It is well-documented that CCB affect blood Tacrolimus concentrations; a large reduction in tacrolimus first-pass metabolism and postabsorptive clearance led to a dramatic increase in tacrolimus blood levels.[28,29] Previous studies found that the CL/F of tacrolimus correlated well with PTD. Antignac *et al*[16] reported that the CL/F of tacrolimus increases with increasing postoperative days until a plateau is reached 2 months post-transplantation. On the other hand, Staatzet al.[15] and Zhang *et al*[30] reported that CL/F inversely correlated with PTD. In the present study, we attempted to use numerous approaches to demonstrate the effect of PTD on CL/F, but no significant change (p>0.001) in the OFV was obtained. Thus, PTD was not included in the final model. Many authors have demonstrated that the clearance of tacrolimus correlates with PTD, maybe the small group of patients of our analysis was not enough to establish their effect on CL/F. [15,16,19,21,30] It is possible that further investigations based on a much larger database have to be performed to explain this.

It has been reported that one major factor that may be involved in pharmacokinetic behavior in kidney transplantation is the concomitant use of Prednisone.[31] CYP3A4 is the principal enzyme responsible for the metabolism of tacrolimus. Corticosteroid use and its long-term administration lead to the induction of CYP3A4 enzymes, causing an increase in tacrolimus clearance. Controversial studies have identified corticosteroid therapy as a significant factor in tacrolimus CL/F variability.[16,32] Although the use of corticosteroids forms part of the triple immunosuppressive therapy of the transplanted patients at our Institution, inclusion of the Prednisone dose as a covariate in the pharmacokinetic analysis showed an increase of OFV in the basic model; thus, it was not included in the final model.

Mean population V/F in the final model was 812.5 L with an estimated interindividual variability of 82.1%. Similar results have been described by other authors[15,19]. During modeling, there was some difficulty in obtaining reasonable individual estimates of V/F for all patients. In the final population model, estimated interindividual variability in V/F was greater than would normally be expected. This could be attributed to tacrolimus trough concentrations, because better estimates of volume of distribution will be obtained when the samples taken cover a large part of the elimination phase, not only trough concentrations[33]. It appears that given such a sampling situation there was insufficient information available to accurately characterize V/F in all patients.

Part of the interindividual variability found in tacrolimus V/F was explained by the patient's hematocrit value. Our results showed an association of hematocrit with the V/F of Tacrolimus. Tacrolimus is extensively distributed in red blood cells, and the whole blood-to-plasma-ratio range was >30:10 over low-to-high plasma concentrations.[34,35] This variability in the tacrolimusblood:plasma ratio is likely due to interpatient differences in hematocrit (range, 17-64.3%), the concentration-dependent distribution of the drug between blood and plasma, and the drug-binding capacity of erythrocytes. Therefore, changes in hematocrit alter the distribution of tacrolimus.

On the other hand, one limitation of our study is the absence of the inclusion of genetic polymorphisms as covariates. This study was conducted with the use of data that were available as part of routine patient care recorded in clinical histories; unfortunately, the performance of clinical

pharmacogenetic studies are not performed routinely and genotypes data were not parameters monitored by the transplant physicians.

Recently, a prospective study in Mexican patients has provided evidence for individualizing the first oral dose of tacrolimus on the basis of the *CYP3A5* genotype, leading the authors to propose that CYP3A5*3*3 expressors required a significantly lower tacrolimus dose (0.07 mg/kg/day) than those with the CYP3A5*1 allele (0.16 mg/kg/day).[36]

In this study, a relatively large (unexplained) residual variability was obtained. This is probably due to the large intraindividual variability in the tacrolimus pharmacokinetic, interoccasion variability, assay errors, sampling time errors, and model misspecification. Notwithstanding our validation analysis confirmed that the model is robust and stable based. External validation is the most stringent test of a model. The performance (precision and accuracy) of the final model is better than that of the basic one in terms of MPE and RMSE (Table 3). The 95% confidence interval of MPE includes zero, which indicates that the final model fits the observed concentrations well.

CONCLUSIONS

A population pharmacokinetic model of tacrolimus was developed in Mexican adult kidney transplant recipients and their typical population values for pharmacokinetic parameters were estimated. Our results indicated that the use of antihypertensive drugs significantly affects the CL/F and that the hematocrit value significantly affects V/F. More population studies in Mexican patients are needed to establish how determination of the CYP3A5 genetic polymorphism might help to develop rational guidelines for the individualized dosage prediction of tacrolimus. Much of the observed inter-subject variability still remains unexplained; however, application of our model in clinical practice should provide better predictability of drug exposure in the population studied.

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