Investigation of the Impact of CYP3A5 and CYP2C19 Polymorphisms on Drug-Drug Interactions between Tacrolimus and Voriconazole Based on Physiologically-Based Pharmacokinetic Modeling

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Abstract

ABSTRACT Aims: This study aimed to develop a PBPK model for tacrolimus incorporating CYP3A5 and CYP2C19 polymorphisms to predict the DDIs between tacrolimus and voriconazole. Methods: Pharmacokinetic (PK) data in rats and healthy subjects receiving tacrolimus with and without voriconazole were used for model development and evaluation. Then, we used the final model to simultaneously investigate the effect of CYP3A5 and CYP2C19 polymorphisms on the PK data of tacrolimus when combined with voriconazole. Results: The final results showed that the predicted C_{max} in CYP3A5 nonexpressers was 1.5-fold higher than expressers, and the predicted AUC_{0-∞} was 1.92 to 1.96-fold higher in nonexpressers. However, the C_{max} and AUC_{0-∞} of tacrolimus both have no significant difference between different CYP2C19 metabolizers. Conclusions: A physiologically-based pharmacokinetic (PBPK) model for tacrolimus integrated with CYP3A5 and CYP2C19 polymorphisms was successfully established, providing more insights regarding the DDIs between tacrolimus and voriconazole in patients with different CYP3A5 and CYP2C19 genotypes. Furthermore, this study highlights the feasibility of PBPK modeling to predict DDIs between these two drugs and the need to include CYP3A5 polymorphisms but not CYP2C19 polymorphisms.

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What is already known about this subject

* Physiologically-based pharmacokinetic (PBPK) modeling approaches combined with in vitro to in vivo extrapolation techniques have been extensively used for predicting clinically meaningful pharmacokinetic drug interactions.

* The drug-drug interactions (DDIs) between tacrolimus and voriconazole increases the difficulty of tacrolimus dosing in clinic. CYP3A5 and CYP2C19 polymorphisms may affect the magnitude of DDIs. However, there is no PBPK model to simultaneously describe the effect of different CYP3A5 and CYP2C19 genotypes on the DDIs.

What this study adds

* A PBPK model for tacrolimus integrated with CYP3A5 and CYP2C19 polymorphisms was successfully established, providing a tool to evaluate the DDIs between tacrolimus and voriconazole in patients with different CYP3A5 and CYP2C19 genotypes.

* The pharmacokinetics of tacrolimus in CYP3A5 nonexpressers were more susceptible to the concomitant use of voriconazole than in CYP3A5 expressers. When genotypes of CYP3A5 were the same, different genotypes of CYP2C19 had no significant effect on the pharmacokinetics of tacrolimus in combination with voriconazole.

ABSTRACT

Aims: This study aimed to develop a PBPK model for tacrolimus incorporating CYP3A5 and CYP2C19 polymorphisms to predict the DDIs between tacrolimus and voriconazole.

Methods: Pharmacokinetic (PK) data in rats and healthy subjects receiving tacrolimus with and without voriconazole were used for model development and evaluation. Then, we used the final model to simultaneously investigate the effect of CYP3A5 and CYP2C19 polymorphisms on the PK data of tacrolimus when combined with voriconazole.

Results: The final results showed that the predicted C_{max} in CYP3A5 nonexpressers was 1.5-fold higher than expressers, and the predicted $AUC_{0-[\tau]}$ was 1.92 to 1.96-fold higher in nonexpressers. However, the C_{max} and $AUC_{0-[\tau]}$ of tacrolimus both have no significant difference between different CYP2C19 metabolizers.

Conclusions: A physiologically-based pharmacokinetic (PBPK) model for tacrolimus integrated with CYP3A5 and CYP2C19 polymorphisms was successfully established, providing more insights regarding the DDIs between tacrolimus and voriconazole in patients with different CYP3A5 and CYP2C19 genotypes. Furthermore, this study highlights the feasibility of PBPK modeling to predict DDIs between these two drugs and the need to include CYP3A5 polymorphisms but not CYP2C19 polymorphisms.

Keywords: drug-drug interactions, tacrolimus, voriconazole, PBPK modeling, CYP3A5 polymorphisms, CYP2C19 polymorphisms

INTRODUCTION

Combination therapy is common in clinical practice, which can result in pharmacokinetic drug-drug interactions (DDIs). It is usually caused by the interference of a perpetrator drug on the metabolizing enzymes or drug transporters of a victim drug^[1]. Genetic polymorphisms that alter these enzymes or transporters thereby can affect the magnitude of DDIs. For example, the drug interaction between tacrolimus and azole antifungals has been reported to be negligible in renal transplant recipients lacking *CYP3A5*1* allele with no functional enzyme activity^[2, 3].

Tacrolimus, a first-line immunosuppressive agent for organ transplant patients, is commonly prescribed with voriconazole to reduce fungal infections after transplantation. However, the concomitant use of voriconazole contributes to an threefold to tenfold increase on tacrolimus concentration, which is highly variable

among individuals and may increase the toxicity on patients^[4]. DDIs between these two drugs may account for tacrolimus pharmacokinetic (PK) alteration. Tacrolimus is mainly metabolized by CYP3A4 and CYP3A5^[5, 6], while voriconazole is mainly metabolized by CYP3A4 and CYP2C19. Voriconazole is also a strong inhibitor of CYP3A4/5^[7]. The polymorphisms of CYP3A5 account for 40-50% of the variability in tacrolimus dose requirement^[8]. Patients carrying at least one functional CYP3A5 allele (*CYP3A5*1*) require approximately 1.5 to 2-fold higher oral tacrolimus doses than patients homozygous for *CYP3A5*3*^[9-12]. A study demonstrated that CYP2C19 genotypes influenced the exposure of tacrolimus when coadministered with voriconazole, although tacrolimus is mainly metabolized by CYP3A^[4]. Moreover, based on the observational clinical data in solid organ recipients, CYP3A5 expressers were associated with lower changes in concentration-to-dose ratio of tacrolimus after coadministration with voriconazole. And, comparing with CYP3A5 nonexpressers, DDIs between tacrolimus and voriconazole were expected to be smaller in expressers^[13]. However, a study demonstrated that the extent of the interaction was not different between CYP3A5 expressers and nonexpressers^[14]. All of these indicates that the effect of CYP3A5 and CYP2C19 genotypes on DDIs between tacrolimus and voriconazole is worth considering.

In recent years, physiologically based pharmacokinetic (PBPK) models have become a hot topic in researches of DDIs, and PBPK models have been recommended to infer effects of concomitant medication and genetic polymorphism on drug metabolizing enzymes and transporters^[15, 16]. A PBPK model of tacrolimus was developed to assess the covariates contributing to the large PK variability of tacrolimus^[17]. Moreover, Kotaro et al. successfully evaluated the contribution of liver regeneration as well as hepatic and intestine CYP3A5 genotypes on tacrolimus pharmacokinetics utilizing a minimal PBPK model^[18]. However, there is no PBPK model to simultaneously describe the effect of different CYP3A5 and CYP2C19 genotypes on DDIs between tacrolimus and voriconazole. Therefore, in this study we tried to construct a PBPK model for tacrolimus integrating CYP2C19 and voriconazole inhibition on CYP3A4 and CYP3A5, which can be used to investigate the effect of different CYP3A5 and CYP2C19 polymorphisms on tacrolimus PK when it was in combination with voriconazole.

METHODS

In vitro assays and pharmacokinetic experiments in rats

In our previous study, different concentrations of tacrolimus were incubated with recombinant CYP3A5 enzyme and different concentrations of voriconazole. Then, the inhibitory effect of voriconazole on tacrolimus metabolism mediated by CYP3A5 was assessed in vitro. Moreover, different doses of voriconazole (0, 20 mg/kg) and tacrolimus (1.5 mg/kg) were administered to rats orally or intravenously. Blood samples were collected to detect tacrolimus concentrations. Then, the drug time curve and tacrolimus PK parameters were obtained to investigate the effects of voriconazole on tacrolimus pharmacokinetics.

PBPK model development

The PBPK model for tacrolimus was developed by combining bottom-up and top-down approaches^[19]. The training dataset for model development was obtained from our rat experiments. The clinical trial datasets were utilized for model evaluation. An extensive literature search was performed to obtain (a) drug physiochemical properties; (b) PK parameters describing absorption, distribution, metabolism and excretion processes; and (c) clinical studies of intravenous and oral administration of tacrolimus to healthy subjects with different dosing regimens. The clinical studies were screened and selected according to the following criteria: (1) tacrolimus was administered alone and in combination with voriconazole; (2) healthy volunteers; (3) blood concentration-time datasets of tacrolimus were available; (4) the concentration of tacrolimus was measured by LC-tandem mass spectrometry (LC-MS/MS) method.

The modeling software PK-Sim® (version 9.1.0, part of the Open Systems Pharmacology (OSP) suite) was used for model development, which consists of a system and drug-dependent component. Drug-specific physicochemical properties were obtained from the literature. System-dependent physiological parameters (organ volumes, blood flow rates, hematocrit, etc.) were provided in PK-Sim(r) with the small molecule model^[20-22]. Organ-plasma partition coefficient of tacrolimus was determined using PK-Sim standard method

based on preclinical data. Organ-plasma partition coefficient of voriconazole were determined using the Poulin and Theil method. Demographic characteristics of subjects were taken from each clinical study.

For model development, a simplified assumption was made that the metabolism of voriconazole is mediated exclusively by CYP3A4 and CYP2C19; the metabolism of tacrolimus is mediated by CYP3A4 and CYP3A5. Tissue expression distribution of enzymes was provided by the PK-Sim(r) expression database based on reverse transcription-polymerase chain reaction (RT-PCR) profiles^[23], and the reference value of CYP3A4 was 4.32 $\mu\text{mol/L}$. The concentration of CYP3A5 and CYP2C19 in liver tissue were set to be 3.25^[24] and 0.76^[25] $\mu\text{mol/L}$, respectively. The metabolism process of tacrolimus was described using Michaelis-Menten kinetics, and the main metabolites were 13-demethyl tacrolimus (13-DMT) and 12-hydroxyl tacrolimus (12-HT)^[26]. According to the study of Dai et al^[27], CYP3A4 and CYP3A5 Michaelis constant (K_m) for 12-HT were set to 0.29 and 0.35 $\mu\text{mol/L}$, respectively, and CYP3A4 and CYP3A5 K_m for 13-DMT were both 0.21 $\mu\text{mol/L}$. CYP3A4 and CYP3A5 catalytic number (K_{cat}) for 12-HT were fixed to 0.61 and 1.4 per minute, respectively, and for 13-DMT were 8 and 17 per minute, respectively. The metabolism of voriconazole was defined by Michaelis-Menten kinetics^[28]. As reported by Damle et al.^[29], K_m for CYP3A4 and CYP2C19 was set to 15 and 3.5 μM , respectively, and K_{cat} for CYP2C19 was fixed to 1.19 per minute. CYP3A4 K_{cat} was set to be 2.12 per minute, which was optimized by Li et al^[25]. Voriconazole exhibited a mechanism-based inhibition (MBI) on CYP3A4. Basing on the study^[25], maximum inactivation rate constant (K_{inact}) and K_i (the inhibition concentration when reaching half of K_{inact}) for CYP3A4 were set to 0.015 per minute and 9.33 $\mu\text{mol/L}$, respectively. The K_i for CYP3A5 was set to 1.76 $\mu\text{mol/L}$, which was from the results of our in vitro study.

In order to further improve the accuracy of model fitting, the specific intestinal permeability, blood cells to plasma permeability and permeability from plasma to blood cells of tacrolimus were optimized, including both intravenous and oral administration of tacrolimus.

PBPK model evaluation

During the evaluation of rat-based PBPK model, the predicted blood concentration-time profiles were compared to the data observed in our rat experiments. In addition, the model performance was evaluated by comparison of predicted to observed $AUC_{0-[?]}$ and C_{max} values. The quantitative evaluation criterion for a good model performance was that the ratios of simulated to observed $AUC_{0-[?]}$ and C_{max} should be within 0.5 to 2.0-fold limits.

After the rat-based PBPK model was built up successfully, it was scaled to whole-body PBPK model of human. And the datasets from three clinical trails^[14, 30] were used to evaluate the performance of human PBPK model. The model performance was evaluated using two methods. One was to compare the ratio of predicted versus observed data (C_{max} and $AUC_{0-[?]}$), the other was to describe the 95% population prediction intervals, the visual criteria for a good model performance was that 95% population prediction intervals should cover the observed individual blood concentration-time datasets.

Virtual population characteristics

Based on the demographic characteristics from 3 screened clinical trials, virtual populations of 100 individuals were generated to quantitatively assess the variability of the predicted concentration-time datasets from the respective clinical trials. The information of age, body weight, body height and proportion of female participants was integrated into PK-Sim® for each clinical trial. The coefficient of variation for CYP3A4 and CYP3A5 expression were 41% and 65%, respectively.

Model applications

Different CYP3A5 genotype groups, i.e. CYP3A5 expressers (*CYP3A5* *1 -carrying genotype groups) and nonexpressers (*CYP3A5* *3/*3 genotype groups) were simulated to depict the polymorphisms of CYP3A5 on the metabolism of tacrolimus. The references in liver tissue of CYP3A5 expressers and nonexpressers were set to 3.25 $\mu\text{mol/L}$ and 1.63 $\mu\text{mol/L}$, respectively. Moreover, the different CYP2C19 genotypes were integrated into the model for intermediate metabolizers (IMs; *1/*2, *1/*3, *2/*17, and *2/*2/*17), rapid

metabolizers (RMs; *1/*17, *17/*17), normal metabolizers (NMs; *1/*1) and poor metabolizers (PM; *2/*2, *2/*3, *3/*3), with the reference values of 0.79, 0.76, 0.40 and 0.01 $\mu\text{mol/L}$, respectively^[25]. Based on the PBPK model, we then explored the performance of tacrolimus in combination with voriconazole in different CYP3A5 and CYP2C19 genotype groups.

RESULTS

In vitro assays and pharmacokinetic experiments in rats

From the in vitro enzymatic experiments, we can learn that voriconazole exhibited a competitive inhibition on CYP3A5 and the K_i was 1.76 $\mu\text{mol/L}$ (Table 1). The blood concentrations of tacrolimus in different dosing regimens were shown in Figure 1, and the relevant pharmacokinetic parameters (C_{max} and $AUC_{0-[\tau]}$) were presented in Table 2.

Clinical studies

Among the three concentration-time datasets of tacrolimus from clinical trials, all was used for human PBPK model evaluation. The participants were all healthy volunteers, with an age range of 18-65 years. Administration protocols included both oral and intravenous routes, used alone and in combination.

Model development and evaluation

The physicochemical and pharmacokinetic parameters of tacrolimus and voriconazole are listed in Table 1. During the development of rat-based PBPK model, the specific intestinal permeability, blood cells to plasma permeability and permeability from plasma to blood cells of tacrolimus were optimized to 4.57×10^{-4} cm/min, 0.02 cm/min and 0.41 cm/min, respectively. The ratios of simulated to observed $AUC_{0-[\tau]}$ and C_{max} were within 0.5 to 2.0-fold limits, as shown in Table 2. The observed data from all the rat experiments exhibited a good fit of the predicted concentration-time profiles (Fig. 1).

After scaling to human PBPK model, The predicted PK results for the respective clinical trials in comparison with the observed aggregate values are presented in Table 3, together with administration protocols and subjects' demography. Prediction performance of the model was quantitatively evaluated by the ratios of predicted versus observed aggregate AUC and C_{max} values, which was shown in Table 3. From the three test datasets, all AUC ratios and C_{max} ratios were within 0.5- to 2.0-fold (Table 3). And, all the observed data from clinical trails were in a good fit of their respective predicted profiles (Fig. 2). For a further evaluation, the 95% prediction interval covered the variability of all observed individual data sufficiently (Fig. 2), indicating that simulations grouped by various dosing regimens was suitable to describe effects of dosing alone and in combination on tacrolimus PK.

Model application

The final PBPK model was further applied to predict pharmacokinetics of tacrolimus in people with different genotypes of CYP3A5 and CYP2C19. The results were shown in Figure 3 and Table 4. And the ratios of predicted versus observed aggregate AUC and C_{max} values were within 2.0-fold. In the combination therapy group, when the CYP2C19 genotype was the same, the predicted C_{max} in CYP3A5 nonexpressers was 1.5-fold higher than expressers, and the predicted $AUC_{0-[\tau]}$ was 1.92 to 1.96-fold higher in nonexpressers. However, when CYP3A5 genotype was the same, C_{max} of different CYP2C19 genotypes were basically unchanged, and the $AUC_{0-[\tau]}$ increased slightly with the decrease of CYP2C19 expression activity, which was not significant.

DISCUSSION

We developed a PBPK model for tacrolimus with voriconazole incorporating CYP3A4, CYP3A5 and CYP2C19 activity. Thereafter, the model was used to quantitatively predict the effect of different CYP3A5 and CYP2C19 genotypes on tacrolimus PK when it was combined with voriconazole. We firstly developed and evaluated a tacrolimus PBPK model in rats to gain confidence in the structural model before scaling

to humans. The simulated tacrolimus C_{max} and $AUC_{0-[\infty]}$ for rats and humans were all within two folds of observed values, suggesting reasonable agreement between our model predictions and observations.

Prior pharmacokinetic studies have noted that voriconazole increased concentration of tacrolimus by inhibiting CYP3A4/5 enzymes. Polymorphisms in the CYP3A5 gene explain 40-50% of the variability in tacrolimus requirement^[31, 32]. The hottest single nucleotide polymorphism (SNP) studied most in CYP3A5 is *CYP3A5*3*, and people with CYP3A5*3 has a weak metabolism ability of tacrolimus^[33]. In Chinese, the frequency of *CYP3A5*3* allele is as high as 77.8%, which may be an explanation for the lower dosage required for Chinese people^[34]. For people with *CYP3A5*1* (CYP3A5 expressers), they are advised to increase starting dose to 1.5-2 times of recommended starting dose. And nonexpressers should initiate therapy with standard recommended dose^[35]. In our predicted results, the C_{max} in CYP3A5 nonexpressers was 1.5-fold higher than expressers, which was in accordance with the recommendations. In addition, CYP2C19 polymorphisms have an effect on the metabolism of voriconazole, which may influence the PK of tacrolimus when they were combined^[4, 25]. However, our results show that CYP2C19 polymorphisms has no significant effect on tacrolimus PK.

Furthermore, the manufacturer's guidelines recommend a reduction of tacrolimus dosage by two-thirds when it was in combination with voriconazole^[36]. However, genetic polymorphism increases the individual difference in tacrolimus concentration after combination with voriconazole, and clinical experience shows that the two-thirds reduction in tacrolimus dose causes insufficient drug efficacy for most individuals^[13, 37]. At present, there is no definitive guideline for the combination therapy of tacrolimus and voriconazole. In clinic, tacrolimus is usually administered at a conventional dose, which was then adjusted according to continuous therapeutic drug monitoring (TDM). Such dose adjustment process often takes a long time and has a great impact on the therapeutic effect and life quality of patients after transplantation^[38]. A number of studies used PBPK model to investigate the effect of enzyme genotype on drug PK when drugs were used alone or in combination^[1, 25, 39, 40]. In addition, patients who received a CYP3A5 genotype-based tacrolimus dose needed significantly less time and fewer dose adaptations to reach target, which would be helpful in some potential ways for the patients^[34, 41-43]. Therefore, it is necessary to take the genotype of metabolic enzymes into consideration during the construction of a PBPK model. Our results of simulation demonstrated that voriconazole could increase the exposure of tacrolimus. And, the effect is more significant in CYP3A5 nonexpressers, which indicates that nonexpressers require further reduction in dosage^[44]. The increased susceptibility of nonexpressers to DDIs may be attributed to the stronger inhibition of voriconazole on CYP3A4^[45]. However, incidences of CYP3A4 mutations in Chinese subjects are rare^[46].

Applying the prediction framework of DDIs concluded from our current work could possibly serve as a dosing guidance tool for tacrolimus. However, there are limitations that warrant further discussion. First, all the clinical data were come from healthy volunteers, and different pathophysiological conditions between healthy subjects and transplant patients could alter the drugs' disposition^[47]. Furthermore, the clinical data of tacrolimus considering the genotypes of CYP3A5 and CYP2C19 was limited. Therefore, further investigation should consider specific pharmacokinetic characteristics of transplant patient population. Second, we did not take into account the dosage form of oral tacrolimus during the treatment with voriconazole. Andrew et al. has found out that pro-longed tacrolimus is less susceptible to interaction with voriconazole in healthy volunteers^[14]. Third, our prediction results require further validation from more real-world data to implement the findings.

CONCLUSIONS

It is critical to incorporate CYP3A5 polymorphisms into PBPK models to accurately predict CYP3A5 mediated drug distribution and DDI potential. We have firstly and successfully developed a PBPK model of tacrolimus with voriconazole incorporating different genotypes of CYP3A5 and CYP2C19. In the future, PBPK model can be integrated with TDM to provide a more convenient and efficient method for the individualized medication of tacrolimus.

Competing Interests

There are no competing interests to declare.

Contributors

F.G. drafted the manuscript. Y.Z., J.F.H. designed the research and reviewed the manuscript. F.G., Y.Y.O., Z.Z.L., Y.K., Q.X.L. performed the basic pharmacology research. F.G., Q.X.L. performed the simulation and analyzed the data.

Data Available Statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Figure legends

Figure. 1. Prediction performance of the rat-based PBPK model on tacrolimus blood concentrations in different dosing regimens. Observed data derived from our rat experiments are shown as dots, a different color represents a rat; and the shaded areas illustrate the 95% population prediction intervals. Predicted versus observed PK parameters are summarized in Table 2. *IV* intravenously, *PO* oral, *TAC* tacrolimus, *VOC* voriconazole.

Figure. 2. Prediction performance of the human PBPK model on individual blood concentrations of tacrolimus for different dosing regimens. The observed individual data of 2A and 2C, 2B are shown as dots and they are reported in the literature [30] and [14], respectively. population simulation medians are shown as lines; and the shaded areas illustrate the 95% population prediction intervals. Details of dosing regimens, study populations, and predicted versus observed PK parameters are summarized in Table 3. *IV* intravenously, *PO* oral, *TAC* tacrolimus, *VOC* voriconazole.

Figure. 3. Prediction performance of the PBPK model on individual blood concentrations of tacrolimus in different CYP3A5 and CYP2C19 genotypes for the combination of tacrolimus and voriconazole. Observed individual data reported in the literature are shown as dots [14]; population simulation medians are shown as lines; and the shaded areas illustrate the 95% population prediction intervals. Details of dosing regimens, study populations, and predicted versus observed PK parameters are summarized in Table 3 and 4. *D*, day of treatment, *PO* oral, *RM* rapid metabolizers, *NM* normal metabolizers, *IM* intermediate metabolizers, *PM* poor metabolizers.

Tables

Table 1. Physicochemical and PK parameters of tacrolimus and voriconazole. K_m , Michaelis-Menten constant, which describes the interaction of substrate and enzyme in the absence of inhibitor; K_{cat} , catalytic number; K_{inact} , maximum inactivation rate constant; K_i inhibition constants; $\log P$, logarithmic of octanol-water partition coefficient; pK_a , negative logarithmic of the acid dissociation constant; V_{max} , maximal rate of metabolism.

Table 2. Comparison of the observed and simulated $AUC_{0-[\infty]}$ and C_{max} for rat-based PBPK model. C_{max} , maximal concentration; $AUC_{0-[\infty]}$, area under the curve from 0 to infinity; *IV*, intravenous; *PBPK*, physiologically-based pharmacokinetic; *PO*, taken orally; *sig*, single dose; *D*, day of treatment.

Table 3. Clinical studies with information on dosing regimens, populations, and predicted and observed $AUC_{0-[\infty]}$ and C_{max} ratios. C_{max} , maximal concentration; $AUC_{0-[\infty]}$, area under the curve from 0 to infinity; *sig*, single dose; *Bid*, twice daily; *PO*, oral administration; *IV*, intravenous injection; *D*, day of treatment; *Ratio*, Simulated/observed.

Table 4. Clinical DDI study with information on the CYP3A5 and CYP2C19 genotype used for model application. C_{max} , maximal concentration; $AUC_{0-[\infty]}$, area under the curve from 0 to infinity; *IV*, intravenous; *sig*, single dose; *Bid*, twice daily; *PO*, oral administration; *D*, day of treatment; *Ratio*, Simulated/observed; *RM*, rapid metabolizers; *NM*, normal metabolizers; *IM*, intermediate metabolizers; *PM*, poor metabolizers.

Table 1. Physicochemical and PK parameters of tacrolimus and voriconazole

Parameter	Tacrolimus	Source	Voriconazole	Source
Molecular weight (g/mol)	804.02	Drug label	349.31	Drug label
Binding rate of plasma protein (%)	1.3	[48]	42	[28]
Log P	5.59	ChemAxon predicted	1.8	[28]
pKa	Neutral	Drug label	2.27 (base)	ChemAxon predicted
Solubility (PH) (mg/ml)	0.012 (7.0)	[49]	0.1 (7.0)	ALOGPS predicted
Partition coefficients	PK-Sim standard	PK-Sim standard	Poulin and Theil	Poulin and Theil
Cellular permeabilities	PK-Sim standard	PK-Sim standard	PK-Sim standard	PK-Sim standard
CYP3A4 13-DMT K_m ($\mu\text{mol/L}$)	0.21	[26]	-	-
CYP3A4 13-DMT K_{cat} (1/min)	8	[26]		
CYP3A4 12-HT K_m ($\mu\text{mol/L}$)	0.29	[26]		
CYP3A4 12-HT K_{cat} (1/min)	0.6	[26]		
CYP3A5 13-DMT K_m ($\mu\text{mol/L}$)	0.21	[26]		
CYP3A5 13-DMT K_{cat} (1/min)	17	[26]		
CYP3A5 12-HT K_m ($\mu\text{mol/L}$)	0.35	[26]		
CYP3A5 12-HT K_{cat} (1/min)	1.4	[26]		
CYP3A4 K_m ($\mu\text{mol/L}$)	-	-	15	[28]
CYP3A4 K_{cat} (1/min)			2.12	[29]
CYP2C19 K_m ($\mu\text{mol/L}$)			3.5	[28]
CYP2C19 K_{cat} (1/min)			1.19	[28]
CYP3A4 K_i ($\mu\text{mol/L}$)			9.33	[29]
CYP3A4 K_{inact} (1/min)			0.015	[29]
CYP3A5 K_i ($\mu\text{mol/L}$)			1.76	In vitro assay

Table 2. Comparison of the observed and simulated $AUC_{0-[\infty]}$ and C_{max} for rat-based PBPK model

Dosing regimen	Tacrolimus C_{max} ($\mu\text{mol/L}$) Simulated	Tacrolimus C_{max} ($\mu\text{mol/L}$) Observed	Tacrolimus C_{max} Ratio
Tacrolimus, 1.5mg/kg, sig, IV	-	-	-
Voriconazole, 20mg/kg, sig, PO (D1-D3) + Tacrolimus, 1.5mg/kg, sig, IV (D3)	-	-	-
Tacrolimus, 1.5mg/kg, sig, PO	2.05	1.60	1.42
Voriconazole, 20mg/kg, sig, PO (D1-D3) + Tacrolimus, 1.5mg/kg, sig, PO (D3)	3.79	4.06	0.93

Table 3. Clinical studies with information on dosing regimens, populations, and predicted and observed $AUC_{0-[\infty]}$ and C_{max} ratios

Dosing regimen	Route	n	Male (%)	Age (years)	Weight (kg)
Tacrolimus, 5 mg, sig	PO	12	58	44.6 ± 19.1	-
Tacrolimus, 0.015 mg/kg, sig	IV	12	67	32.2 ± 10.8	-
Tacrolimus, 3 mg, sig + Voriconazole 200 mg, bid, D2-4 (400 mg. Bid, D1)	PO	18	100	18 - 65	[?] 40

Table 4. Clinical DDI study with information on the CYP3A5 and CYP2C19 genotype used for model application

Dosing regimen	CYP3A5 genotype	CYP2C19 genotype	Tacrolimus C _{max} (μmol/L) Simulated	Tacrolimus C _{max} (μmol/L) Observed	Tacrolimus C _{max} (μmol/L) Ratio	Tacrolimus AUC _{0-∞} (μmol*min/L) Simulated	Tacrolimus AUC _{0-∞} (μmol*min/L) Observed	Tacrolimus AUC _{0-∞} (μmol*min/L) Ratio	Source of data
Tacrolimus, 3 mg, sig + Voriconazole 200 mg, bid, D2-4 (400 mg, bid, D1), PO	Expressers (*1/*1, *1/*3)	RM (*1/*17, *17/*17)	0.04	-	-	37.67	-	-	[14]
		NM (*1/*1)	0.04	0.04	1.00	37.69	35.00	1.08	
		IM (*1/*2, *1/*3, *2/*17, *2/*2/*17)	0.04	-	-	38.10	-	-	
	Nonexpressers (*3/*3)	PM (*2/*2, *2/*3, *3/*3)	0.04	-	-	38.66	-	-	
		RM (*1/*17, *17/*17)	0.06	-	-	72.51	-	-	
		NM (*1/*1)	0.06	0.05	1.20	72.60	49.70	1.46	
		IM (*1/*2, *1/*3, *2/*17, *2/*2/*17)	0.06	-	-	73.92	-	-	
		PM (*2/*2, *2/*3, *3/*3)	0.06	-	-	75.65	-	-	

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