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ORIGINAL RESEARCH

Wuzhi Capsule Dosage Affects Tacrolimus Elimination in Adult Kidney Transplant Recipients, as Determined by a Population Pharmacokinetics Analysis

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Purpose: In this study, we aimed to establish a tacrolimus population pharmacokinetic model and better understand the drug-drug interaction between Wuzhi capsule and tacrolimus in Chinese renal transplant recipients.

Patients and Methods: We performed a population pharmacokinetic analysis using a nonlinear mixed-effects model to determine the suitable Wuzhi capsule dose in combination with tacrolimus. Data on 1378 tacrolimus steady-state concentrations were obtained from 142 patients who received kidney transplant in Changhai Hospital and Huashan Hospital. Demographic characteristics, laboratory tests, genetic polymorphisms, and co-medications were evaluated.

Results: The one-compartment model best described data. Our final model identified creatinine clearance rate, hematocrit, Wuzhi capsule dose, *CYP3A5*3* genetic polymorphisms, and tacrolimus daily dose as significant covariates for tacrolimus clearance, with the value of 14.4 L h^{-1}, and the between-subject variability (BSV) was 25.4%. The Wuzhi capsule showed a dose-dependent effect on tacrolimus pharmacokinetics, demonstrating a stronger inhibitory effect than inductive effect.

Conclusion: Our model can accurately describe population pharmacokinetics of tacrolimus when combined with different doses of Wuzhi capsule. Additionally, this model can be used for individualizing tacrolimus dose following kidney transplantation.

Keywords: renal transplantation, inhibitory effect, one-compartment model, *CYP3A5*

Introduction

Tacrolimus (FK506) is an efficacious immunosuppressive drug used following solid organ transplant;[1] however, it has a narrow therapeutic range and large between-patient variability. The bioavailability of tacrolimus is low and highly variable (range 4–89%) owing to its poor solubility and extensive first-pass effect.[2] Therapeutic drug monitoring (TDM) is implemented to individualize the dosage of tacrolimus based on the trough concentration (C_0) and to reduce the risks of toxicity and rejection risk.[3] In general, pharmacokinetic parameters reach a steady state approximately 3 d following therapy initiation. Therefore, early identification of factors that can affect pharmacokinetic biomarkers can facilitate the adjustment of tacrolimus dosage. A population pharmacokinetic (popPK) modeling using the Bayesian estimation is commonly used to quantitatively assess the dependency

between a covariate and a pharmacokinetic parameter for identifying inter- and intra-individual variabilities in the estimated pharmacokinetic parameter and the relevant covariate.[4]

Currently, there are numerous popPK models,[5–9] which have been developed to characterize the pharmacokinetics of tacrolimus in adults after kidney transplant. Several factors have been identified to affect tacrolimus pharmacokinetics, including demographic characteristics; *CYP3A5, CYP3A4*, and *ABCB1* genotypes; hematocrit; post-operative days; tacrolimus daily dose; and drug–drug interactions. The tacrolimus doses recommended by the Food and Drug Administration (FDA) are according to patient age, type of transplanted organ, post-transplant day, and immunosuppressive therapy regimens.[10] Tacrolimus is metabolized in the liver by cytochrome CYP3A isoenzymes and excreted primarily through faces (approximately 93%). The *CYP3A5*3* allele affects tacrolimus pharmacokinetics in organ transplant recipients, and thus dose adjustment based on the *CYP3A5* genotypes is necessary.[11,12] The guidelines from Clinical Pharmacogenetics Implementation Consortium (CPIS) have summarized and emphasized the effect of *CYP3A5* genotype variants on inter-individual variabilities in tacrolimus pharmacokinetics.[13] In addition, tacrolimus is a substrate of the transporter p-glycoprotein (P-gp), which is also known as ATP-binding cassette subfamily B member 1 (ABCB1); however, the effect of ABCB1 in the pharmacokinetics of tacrolimus remains controversial.[14,15] Drug–drug interaction is another important factor that affects tacrolimus disposition. PopPK studies have identified the effect of prednisolone and calcium channel blockers on tacrolimus pharmacokinetics.[16–18]

Wuzhi capsule, a traditional Chinese medicine (registration number in China: Z10983013), is often prescribed to alleviate the hepatotoxicity of tacrolimus in China.[19–21] Wuzhi capsule has been reported to increase tacrolimus exposure, leading to further dose reduction of approximately 50% or more.[22,23] Pharmacokinetic studies[24,25] have showed that co-treatment with Wuzhi capsule can reduce the clearance rate, peak concentration, and peak time delay of tacrolimus. In addition, considering that tacrolimus has a narrow therapeutic range, it is important to acquire the target trough concentration.[3] Studies have also shown that long-term pre-treatment with Wuzhi capsule could induce *CYP3A* and *CYP2C* expression;[26,27] hence, determining the dose-effect relationship between Wuzhi capsule and tacrolimus pharmacokinetics and the possible underlying mechanism of action is important.

For this purpose, in this study, we aimed to (i) elucidate the effects of Wuzhi capsule on tacrolimus pharmacokinetics by establishing a population pharmacokinetic model of tacrolimus based on data from transplant recipients in two centers, namely Changhai and Huashan Hospitals, and (ii) determine the drug-drug interaction between Wuzhi capsule and tacrolimus pharmacokinetics and identify the possible action mechanisms.

Materials and Methods

Data

We obtained whole blood trough concentrations (C_0, n = 758) of 90 renal transplant patients at the Organ Transplantation Department of Changhai Hospital, Navy Medical University (Shanghai, China) from March 2016 to September 2016. Blood trough concentration data of 52 adult renal recipients (C_0, n = 620) were also obtained from Huashan Hospital (Shanghai, China) from May 2009 to December 2013.[8]

All patients were followed-up until post-operative day 90, and the average number of blood concentrations per subject was 37. The exclusion criteria included missing required information, dialysis treatment, acute rejection, second transplantation, and switching of immunosuppressive agents. The study was approved by the Ethics Committee of Changhai Hospital (number: CHEC2017-219) and Huashan Hospital (number: 2011–183). All participants, from both centers, provided written informed consent, and the study was conducted in accordance with the Declaration of Istanbul (2018).

Immunosuppressive Regime

Patients in both centers received a triple immunosuppressive regimen containing tacrolimus, mycophenolate acid (MPA), and corticosteroids.

Changhai Hospital: The initial dosage of tacrolimus was 0.05–1.0 $mg \cdot kg^{-1} \cdot d^{-1}$ and then adjusted according to C_0 (10–15 $ng \cdot mL^{-1}$ in the first post-operative month, 8–10 $ng \cdot mL^{-1}$ between 1 and 3 months, and 5–8 $ng \cdot mL^{-1}$ thereafter) to ensure clinical efficacy. The range of MPA dosage was 0.25–1.0 $g \cdot d^{-1}$. Intravenous methylprednisolone was administered at a dose of 500 mg on the operation day and at 20–40 mg daily within 1 week. Intravenous methylprednisolone was then switched to oral prednisone at 10–20 - $mg \cdot d^{-1}$ on days 7–14, which was tapered to 0–10 $mg \cdot d^{-1}$ during the first 3 months. Wuzhi capsule was administered as appropriate: 709 cases, Wuzhi capsule was co-

administered with tacrolimus; 39 cases, 11.25 mg once a day; 498 cases, 11.25 mg twice daily; 21 cases, 11.25mg three times a day, 135 cases, 22.5 mg twice a day; and 16 cases, 22.5 mg three times a day. Only 49 cases did not receive Wuzhi capsule.

Huashan Hospital: The initial dosage of tacrolimus was 0.1–0.15 $mg \cdot kg^{-1} \cdot d^{-1}$, and the doses were adjusted according to C_0: 8–10 $ng \cdot mL^{-1}$ in the first months after surgery, 6–8 $ng \cdot mL^{-1}$ between 1 and 3 months, and 3–7 $ng \cdot mL^{-1}$ thereafter. MPA was orally administered at 0.5 g twice a day for individuals with a body weight (WT) of <50 kg and 0.75 g twice a day for those with a WT of ≥50 kg, with a dosage range of 0.25–1.0 $g \cdot d^{-1}$. Intravenous methylprednisolone 500 mg was administered for 3 d after surgery, and then switched to prednisolone 80 mg on day 4. The prednisolone dose was subsequently reduced by 5–15 $mg \cdot d^{-1}$ within a month, and then tapered to 10 $mg \cdot d^{-1}$ during the first 3 months. No patient was administered Wuzhi capsule.

Sample Collection and Bioanalytical Assay

In Changhai Hospital, Chemiluminescence microparticle immunoassay (CMIA) was performed using an Architect I 1000 system (Abbott Laboratories, Abbott Park, IL, USA) to determine tacrolimus blood concentration. The lower limit of quantitation (LOD) was 1.5 $ng \cdot mL^{-1}$ and the calibration range was between 2.0 and 30 $ng \cdot mL^{-1}$.[28] In Huashan Hospital, enzyme multiplied immunoassay technique (EMIT) was used with a SYVA Viva-Emit 2000 kit (Siemens Healthcare Diagnostics Inc., Germany). The calibration range was 2.0–30 $ng \cdot mL^{-1}$ and LOD was 2 $ng \cdot mL^{-1}$.[29] There were systematic biases between CMIA and EMIT. Thus, to correct these biases, the data from Huashan Hospital were converted to their corresponding equivalents in terms of the reported bioassays using the following formula:[28]

CMIA=0.93*EMIT+0.36.

Genotyping

Blood samples from Changhai Hospital and Huashan Hospital were delivered to Shanghai Yunzhou Biotechnology Co., Ltd. (Shanghai, China) and Huashan Huada Biotechnology Co., Ltd. (Shanghai, China), respectively, for DNA extraction, PCR analysis, and subsequent DNA sequencing. Four single-nucleotide polymorphisms, including *CYP3A5*3* (rs776746), *ABCB1Exon12* (C1236T, rs1128503), *ABCB1Exon21* (G2677A(T), rs2032582), and *ABCB1Exon26* (C3435T, rs1045642), were genotyped. Pearson's x^2 test was conducted to assess the deviation in the frequencies of CYP3A5 and ABCB1 genotypes from the Hardy–Weinberg equilibrium.

Population Pharmacokinetic Modeling

We collected demographic and covariate information from patients' medical records system and TDM database. Nonlinear mixed-effects modeling software was used to conduct this PopPK analysis (NONMEM®, version 7.4; ICON Development Solutions, Ellicott City, MD, USA). The NONMEM data file was created using R (version 3.5.1; www.r-project.org). The final base popPK model was selected after comparison of diagnostic plots, objective function value and Akaike information criterion.

Base Model Development

The popPK of tacrolimus was analyzed using a one-compartment (1-CMT) model with the first-order absorption and elimination with parameters depending on total clearance (CL/F) and volume of distribution (Vd/F). As only the trough concentration was obtained and because there was no sampling in the absorption phase, between-subject variability (BSV) of Ka need not be included in the analysis. The Ka was fixed at 3.09 h^{-1} with no BSV based on the findings of a previous study,[6] which used the same population with a similar Ka value in the NONMEN analysis.

We used an exponential model for all parameters for BSV. The equation (Eq) was as follows (except for Ka):

$$P_i = TV\ (P) \times exp\ (\eta_i) \quad \text{(Eq 1)}$$

where P_i represents the ith subject's pharmacokinetic parameter. $TV\ (P)$ indicates the typical value of the population parameter, and ηi is the symmetrically distributed BSV and zero-mean random variable with a square deviation of ω_i^2.

Residual unexplained variability (RUV) was determined using an additive model, a proportional model, and a combination of proportional and additive models (Eqs. 2–4):

$$F = IPRED + \varepsilon 1 \quad \text{(Eq 2)}$$

$$F = IPRED \times \exp(\varepsilon 1) \quad \text{(Eq 3)}$$

$$F = IPRED \times \exp(\varepsilon 1) + \varepsilon 2 \quad \text{(Eq 4)}$$

where F represents the value of observation, IPRED is the prediction value of individual, and ε_n is the symmetrically distributed variables with variance terms, which were partially estimated using the population model-fitting process.

Covariate Analysis

We estimated the potential covariates in pharmacokinetic parameters, which included weight, height, aspartate aminotransferase level, total serum bilirubin level, hematocrit, creatinine clearance rate, tacrolimus daily dose, postoperative time, and concomitant medications as continuous covariates. *CYP3A5* and *ABCB1* genes, co-therapy with Wuzhi capsule at cumulative doses for 48 h, and co-therapy with corticosteroids at cumulative doses for 24 h as categorical covariates. Only co-medications that constituted >10% of all patients were tested.

We normalized the continuous covariates to the population median values; taking the creatinine clearance rate as an example, the continuous covariates were tested using linear equations (Eqs. 5–6) and power (Eq. 7) as shown below:

$$Pi = TV(P) + \theta_{\mathrm{cov}} \mathrm{x} \left(\frac{COV_i}{COV_{median}} \right) \quad (5)$$

$$Pi = TV(P) + \theta_{\mathrm{cov}} \mathrm{x} (COV_i - COV_{median}) \quad (6)$$

$$Pi = TV(P) \mathrm{x} \left(\frac{COV_i}{COV_{median}} \right)^{\theta \mathrm{cov}} \quad (7)$$

where, Pi represents the i^{th} subject's pharmacokinetic parameter, the typical value for the parameter is $TV\ (P)$, COV_i means the i^{th} individual's covariate value, COV_{median} is the population median value, and θ_{cov} is the coefficient of the covariate effect, which was estimated.

Categorical variables, such as sex, were investigated using a scale model.

Pi=TV(P), if sex=female

where θ represents the degree of influence of a covariate on the parameters.

We used a stepwise forward inclusion and backward elimination strategy. A decrease in the objective value (OFV) of at least 10.83 ($P < 0.001$, $df = 1$) was set as a criterion for inclusion of a covariate in the model in the forward inclusion analysis stage. An increase in OFV of at least 10.83 ($P < 0.001$, $df = 1$) was set as a criterion for retaining parameters for backward elimination. We excluded covariates with an unexplainable pharmacological or biological basis, or a parameter effect of <10%. The covariates in the model should improve precision of the parameter estimation and goodness-of-fit plots and reduce the BSV and RUV. Finally, the shrinkage extent of the final model was evaluated.

Model Validation

Goodness-of-fit plots, which included the observed concentrations (DV), were plotted against population prediction concentration (PRED) and individual prediction concentration (IPRED), and conditional weighted residuals (CWRES) versus PRED and time after dose were also used to evaluate the fitness of the final model. In addition, we also used bootstrap, prediction-corrected visual predictive checks (pc-VPCs), and normalized prediction distribution errors (NPDEs) to evaluate the performance of the final model.

We used non-parametric bootstrap to assess the robustness of the final model. A total of 2000 bootstraps were performed using NONMEM program. The 2.5th to 97.5th percentiles and the medians of the parameters after bootstrap with successful convergence compared with the final model parameter estimates were obtained using NONMEM®.

Shrinkage was assessed for the between-subject random effects (η) and the residual random effect (ε) of the final model. Shrinkage quantifies the quality of information in the dataset to estimate individual parameter estimates. Empirical Bayes estimates should be interpreted with caution when substantial η- or ε-shrinkage >30%.

For pc-VPCs, the final model was simulated 1000 times to produce the expected concentrations in NONMEM. Profiles for concentration-time of the 10th, 50th, and 90th percentiles of the simulated data were generated and combined with the observed data to produce the expected concentrations.

NPDEs were also investigated. Each observation in the original data was simulated 5000 times using the final model parameters with the simulation-based diagnostic. The NPDE results were statistically summarized and displayed graphically using the NPDE add-on package in R.[30]

The NPDE results were summarized graphically using quantile-quantile (Q-Q) plot, histogram scatter plot versus time, and PRED. If the NPDE conformed normal distribution with a mean value of zero and a variance of one, then the predictive performance is considered satisfied.

Simulation of Tacrolimus Dosing Strategies with Different Dosages of Wuzhi Capsules

Monte Carlo simulations were performed to estimate the parameters using the final population pharmacokinetic model. The aim was to find the dosing regimen of Wuzhi capsules that can achieve the target C_0 (10–15 ng·mL^{-1})

after 7 days of multiple oral doses in subjects with different *CYP3A5* genotypes. In the simulations, the individual daily dose of tacrolimus was 4, 6, and 8 mg; the 48-h Wuzhi capsule dose was <45 mg, = 45 mg, and >45 mg; the subjects were *CYP3A5* expressers and non-expressers. We performed 1000 simulations using the initial database and C_0 was calculated for each simulation.

Results

Study Population

A total of 142 kidney recipients were enrolled in this study, and 1378 trough blood tacrolimus concentration data were available for modeling. In Changhai Hospital, the mean dose of tacrolimus was 5.6 $mg \cdot d^{-1}$ and the mean tacrolimus C_0 was 14.8 $ng \cdot mL^{-1}$. In Huashan Hospital, the mean dose of tacrolimus was 4.6 $mg \cdot d^{-1}$ and the mean tacrolimus C_0 was 6.5 $ng \cdot mL^{-1}$. Data of patients' demographics, laboratory test results, and distribution of the *CYP3A5*3, ABCB1Exon12, ABCB1Exon21*, and *ABCB1Exon26* genotypes, which were used for developing the model, are summarized in Table 1. The primer sequences of the CYP3A5*3, ABCB1Exon12, ABCB1Exon21, and ABCB1Exon26 genotypes are presented in Table S1. There was no deviation from Hardy-Weinberg equilibrium ($P > 0.05$). The wild types of *CYP3A5* constituted 8.54% of the total genotypes. Detailed immunosuppressive regimens and co-medications in Changhai Hospital and Huashan Hospital are presented in Table S2. The doses of mycophenolate and glucocorticoid in Huashan Hospital were higher than those in Changhai Hospital, which may explain why the C_0 of tacrolimus was lower in the former than in the latter.

Population Pharmacokinetic Modeling

Our results indicated that a one-compartment model with the first-order absorption was the right choice for modeling. Our final model was stable and predictable and can be used in clinical setting. The residual error model was selected using an exponential method. The model revealed a significant decrease in the OFV for CL/F. None of the covariables affected the OFV of Vd/F in the covariate screening of the Vd/F ($P > 0.001$) as only trough concentrations were available. All covariates were retained in the final model in the backward elimination step. The forward addition and backward elimination results are presented in Table S3. The final popPK model included the following parameter-covariate relations:

$CL/F = 14.4 \times (CCR/45.5)^{0.179} \times (28.9/HCT)^{0.503} \times (DOSE/5)^{0.351} \times 1.29$ (if CYP3A5*1*3 and *1*1 carriers) × 0.566 (if 48-h Wuzhi capsule dosage <45 mg) × 0.783 (if 48-h Wuzhi capsule dosage = 45 mg) × 0.598 (if 48-h Wuzhi capsule dosage >45 mg) × 1 (if 48-h Wuzhi capsule dosage = 0 mg)

$Vd/F = 275$

where CCR is the creatinine clearance rate by Cockcroft-Gault equation, HCT is hematocrit, and DOSE is the daily dose of tacrolimus.

The population parameter estimates of CL/F and Vd/F in the final model were 14.4 $L \cdot h^{-1}$ and 275 L, respectively. Furthermore, we also examined the effect of changes in Ka on CL/F. CL/F only decreased from 14.2 to 13.6 when Ka increased from 1 h^{-1} to 10 h^{-1}, showing a change rate of less than 5%. All retained covariates in the final model explained the 25.4% BSV in CL/F compared with those in the base model, and the creatinine clearance rate showed an obvious effect on CL/F. Moreover, concomitant therapy with Wuzhi capsule led to >20% lower CL/F than non-concomitant therapy. The shrinkage for CL/F was estimated to be 12% and 25% for Vd/F. Precision of all parameters represented by a standard error was acceptable (<30%). The estimation of the population pharmacokinetic parameter and the accuracy of the final model with covariates are presented in Table 2.

Model Validation

Goodness-of-Fit Plots

The goodness-of-fit plots for the base and final models are shown in Figure 1 (a1 and a2). There were no significant biases and deviations in the final model, and the fitting of the final model was greatly improved compared with that of the base model. In the scatterplot of PRED versus DV, there were some underpredictions at high concentrations. However, CWRES were within ± 2, revealing the final model had acceptable fitness.

Bootstrap Analysis

The data were resampled and 95.6% of 1000 runs were successful in the bootstrap. The median and confidence interval between 2.5% and 97.5% of the estimated values of pharmacokinetic parameters obtained from bootstrap were close to the original dataset with <5% bias. Therefore, the final model was confirmed to be stable with good precision of parameter estimation, as shown in Table 2.

Table 1 Demographic Data of the Patients After Kidney Transplantation

Characteristic	Changhai Hospital		Huashan Hospital		Total	
	Mean ± SD	**Median (Range)**	**Mean ± SD**	**Median (Range)**	**Mean ± SD**	**Median (Range)**
Number of patients (M/F)	90 (61/29)	/	52 (35/17)	/	142 (96/46)	/
Number of observed samples	758	/	620	/	1378	/
Ages (years)	42.2±11.5	41.00 (20.0–67.0)	38.5±9.1	39.0 (21.0–60.0)	40.9±10.8	40.0 (20.0–67.0)
Body weight (kg)	61.2±10.0	61.2 (36.0–86.7)	59.4±10.3	60.0 (40.0–83.0)	60.5±10.1	60.1 (36–86.7)
Height (cm)	168.4±6.8	169.0 (150.0–182.0)	167.6±7.0	169 (150–180)	168.1±6.8	169.0 (150–182.0)
Haematocrit (%)	29.0±5.5	28.7 (18.3–48.2)	29.7±5.2	29.8 (17.6–46.2)	29.2±5.4	28.9 (17.6–48.2)
Total protein(g/L)	64.6±7.8	65 (43–89)	66.8±12.0	66 (26–89)	65.4±9.6	65 (26–89)
POD	15.1±13.8	11.0 (2–87)	37.0±25.9	31 (2–90)	24.9±22.9	16 (2–90)
AST (U/L)	16.2±8.0	15.0 (6.0–71.0)	27.3±29.7	16.0 (8.0–187.0)	20.3±19.7	15.0 (6.0–187.0)
SCR (μmol/L)	308.3±315.5	170.0 (51.0–1397)	170.3±114.8	131.5 (49.0–710.0)	260.2±268.6	165.5 (49.0–1397)
CCR (mL/min)	42.6±26.8	41.5 (4.9–123.9)	54.3±25.8	52.1 (10.7–108.8)	46.6±27.0	45.5 (4.9–123.9)
FK506 daily dose (mg/days)	5.6±1.7	6 (2–11)	4.6±1.8	5 (1–8)	5.2±1.8	5 (1–11)
FK506 concentration (ng/mL)	14.8±5.3	14.2 (3.2–42.6)	6.5±2.8	6.22 (2.3–24.7)	11.10±6.0	9.9 (2.3–42.6)
Co-therapy medications						
Wuzhi capsule [a]	90 (709)	/	/	/	90 (709)	/
24h Wuzhi capsule dosing	/	22.5 (0–67.5)	/	/	/	/
48h Wuzhi capsule dosing	/	45 (0–135.0)	/	/	/	/
Oral prednisone daily dose(mg/ day)	/	10 (0–640)	/	/	20 (5–655)	/
Concomitant calcium channel	51 (432)	/	37 (520)	/	88 (952)	/
Concomitant Diltiazem	26 (175)	/	/	/	/	/
Bailing Capsule	61 (479)	/	/	/	/	/
Genotypes[a]	Number of patients					
CYP3A5*3(AA/AG/GG)	8/29/53	/	4/19/29	/	12/48/82	/
ABCB1Exon12(CC/CT/TT)	14/36/40	/	6/19/27	/	20/55/67	/
ABCB1Exon21(GG/TG/TT)	19/46/25	/	4/19/29	/	33/69/40	/
ABCB1Exon26(CC/CT/TT)	39/40/11	/	14/23/15	/	64/64/14	/

Notes: [a]All frequencies were in agreement with those predicted by Hardy-Weinberg equation ($P > 0.05$).

Abbreviations: POD, Postoperative time; AST, Alanine transaminase; SCR, serum creatinine; CCR, calculated based on serum creatinine using Cockcroft-Gault equation as follow: CCR=[140-Age (years)]*Body weight (kg)/[0.818*SCRμmol/L)]× (0.85, If the patient is female).

Table 2 Population Pharmacokinetic Parameter Estimates of the Base Model, Final Model, and Bootstrap of the Final Model

Parameter	Base Model	Final Model	Bootstrap of Final Model (n=1000)	
	Estimate(RSE %) [Shrinkage%]	Estimate(RSE %) [Shrinkage%]	Median	2.5%-97.5%
Objective function value(OFV)	4999.679	4577.955	4547.469	4144.916–4966.967
Ka* (h−1)	3.09	3.09	3.09	/
CL/F (L/h)	13 (3)	14.4(7)	14.30	11.84–16.38
Creatinine clearance rate	/	0.179(18)	0.176	0.097–0.236
Haematocrit	/	0.503(14)	0.508	0.385–0.657
CYP3A5*1/*1 and*1/*3	/	1.29(5)	1.288	1.160–1.424
48h cumulative dosage of Wuzhi Capsule(WZ)	/	/	/	/
WZ<45mg	/	0.566(27)	0.575	0.366–0.810
WZ=45mg	/	0.783(8)	0.778	0.671–0.921
WZ>45mg	/	0.598(6)	0.6	0.527–0.691
WZ=0mg	/	1	/	/
DOSE	/	0.351(13)	0.343	0.250–0.432
Vd/F (L)	259 (6)	275(29)		
Between-subject variability	/	/	/	/
CL/F(%)	34.8(8)[17]	25.4(15)[12]	24.8	16.70–30.63
V/F(%)	83.6(8)[15]	51.5(35)[25]	50	18.81–62.37
Residual variability	/	/	/	/
CH exponential error	0.0752(10)[8]	0.0606(14)[8]	0.060	0.048–0.075
HS exponential error	0.107(6)[4]	0.0887(8)[4]	0.0887	0.075–0.102

Notes: *Ka was fixed to the published value.
Abbreviations: Ka, Absorption rate constant; CL/F, Apparent clearance; Vd/F, Apparent volume; DOSE, The daily dose of tacrolimus; CH, Changhai Hospital; HS, Huashan Hospital.

Pc-VPC

The pc-VPCs results of the final model are presented in Figure 2. The 10^{th}, 50^{th}, and 90^{th} percentiles of the observations were within the 95% confidence interval (CI) of the final model's corresponding prediction percentiles. In addition, nearly 6.83% of the observations was outside the 90% prediction range, revealing that the simulated data and observed concentrations are acceptable.

NPDE

The results of NPDE are presented in Figure 3. The Q-Q plots and histogram of the final model confirmed that the NPDE was normally distributed and had good predictability. The differences between predictions and observations were acceptable with P values >0.05.

Dosing Selection Strategies

Monte Carlo simulation results are shown in Figure 4. Serum trough concentrations with Wuzhi capsule (<45 mg, = 45 mg, and >45 mg) and without Wuzhi capsule post renal transplantation were simulated. Our modeling data were focused on the first 7-d period post renal transplantation, with target tacrolimus C_0 following renal transplantation of 10–15 $ng \cdot mL^{-1}$. For *CYP3A5 *1* carriers (AA/AG) who were not administered Wuzhi capsule, the current dosage seemed insufficient when compared with that for *CYP3A5 *1* non-carriers. Moreover, to achieve the target concentration, patients co-administered with Wuzhi capsule received a lower dosage than *CYP3A5 *1* non-carriers receiving only tacrolimus (2.0–3.0 mg q12h vs ≥3.0 mg q12h). Wuzhi capsule at an accumulated dose of below 45 mg (48-h) received the lower dosage, with *CYP3A5* non-expressing patients receiving only tacrolimus at 1.0–2.0 mg q12h to attain the target concentration. The model diagnostic diagram indicated a good performance in describing tacrolimus concentration in the period of data collection.

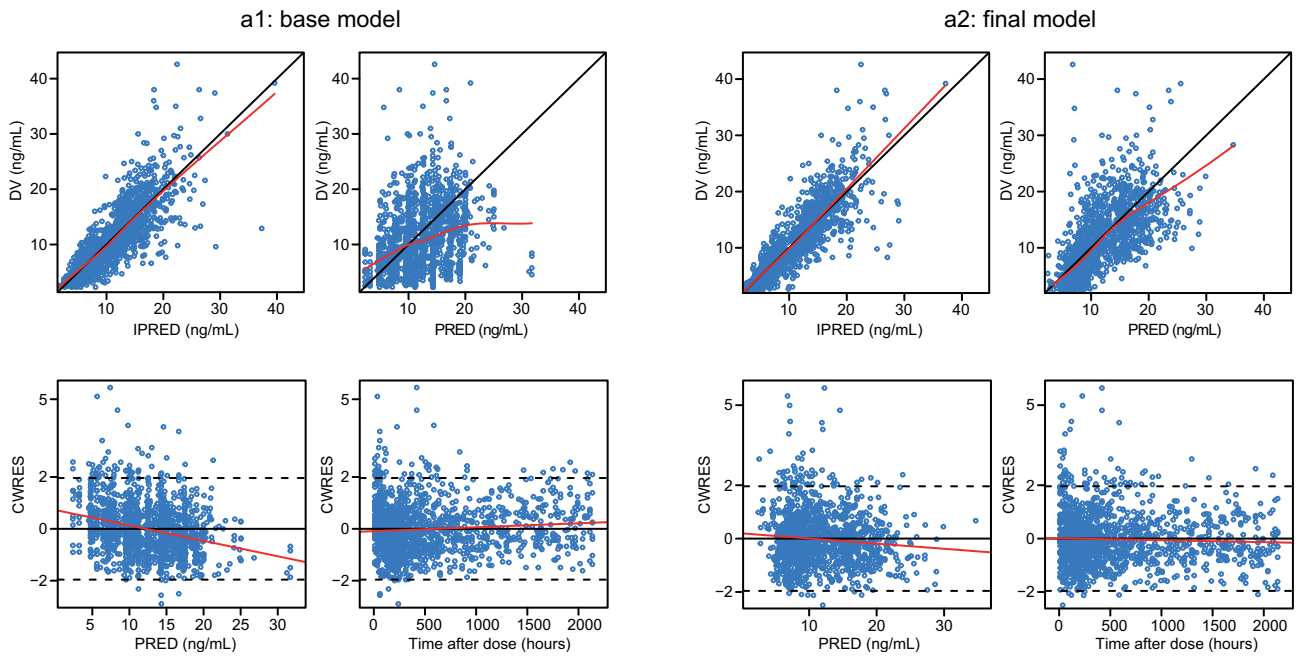

Figure 1 Goodness-of-fit plots for the base model (Figure a1) and final model (Figure a2).
Abbreviations: DV, dependent variable; IPRED, individual prediction; PRED, population prediction; CWRES, conditional weighted residual.

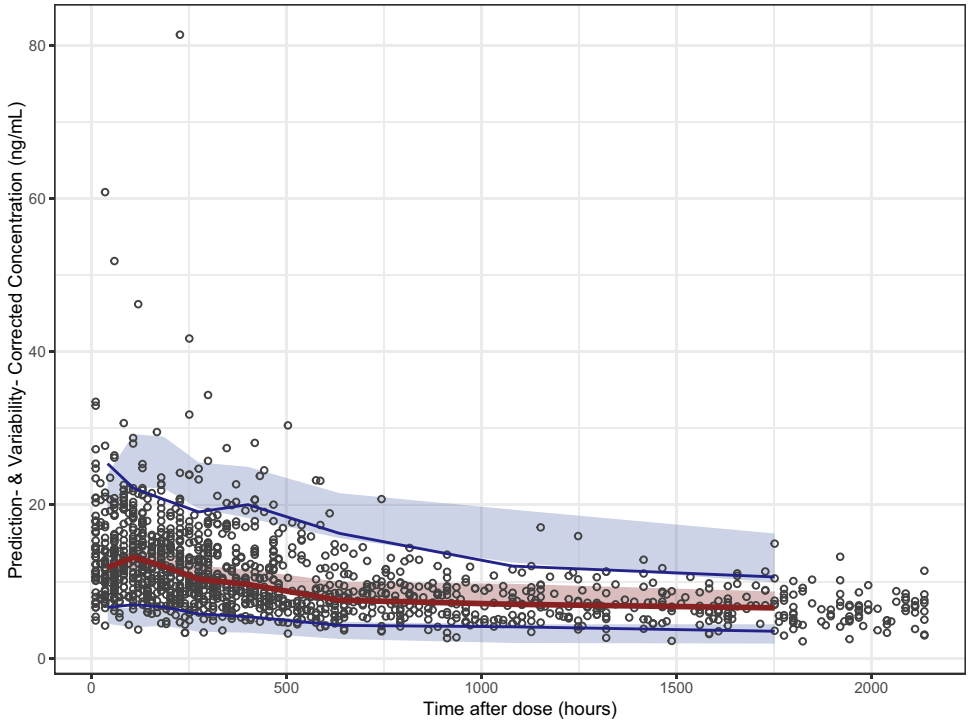

Figure 2 Prediction-corrected visual predictive check (pc-VPC) plot of the final pharmacokinetic model. The red solid line represents the prediction-corrected median observed concentration, and the semitransparent red shaded area represents the simulation-based 95% confidence intervals (CIs) for the median. The blue solid line represent the corrected observed 10th and 90th percentiles and the semi-transparent blue shaded areas represent the simulation-based 95% CIs for the corresponding predicted percentiles from the final model. The black dots represent the prediction-corrected observations.

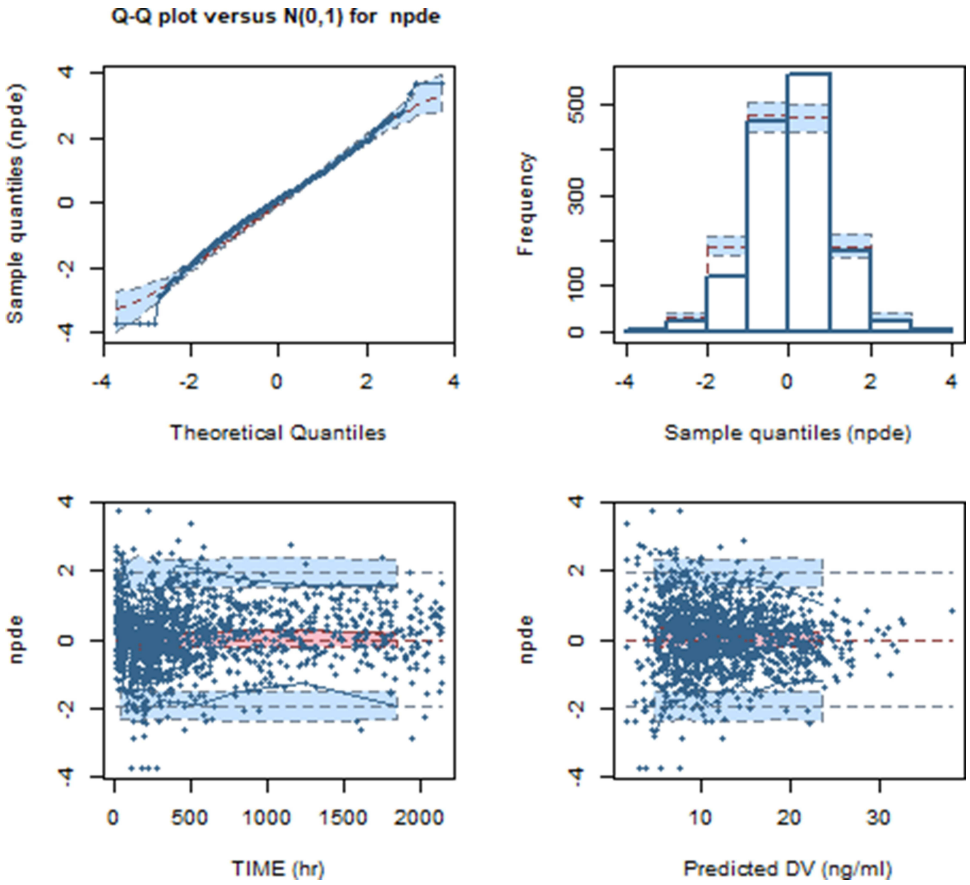

Figure 3 Normalized prediction distribution error (NPDE) plot of the final model. (**A**) Quantile–quantile plot of the distribution of NPDE against the theoretical distribution (semi-transparent blue fields), (**B**) Histogram of the distribution of NPDE against the theoretical distribution (semi-transparent blue fields), (**C**) NPDE vs postoperative time (days), (**D**) NPDE vs predicted concentrations. In plot C and D, the red solid lines represent the median NPDE of the observations, and semi-transparent red fields represent the simulation-based 95% confidence intervals (CIs) for the median. Blue solid lines represent NPDE of the observed 5th and 95th percentiles, and semi-transparent blue fields represent the simulation-based 95% CIs for the corresponding predicted percentiles from the final model. The blue dots represent the NPDE of the observations.

Discussion

To the best of our knowledge, Wuzhi capsule is commonly used in Chinese solid organ transplantation to reduce tacrolimus dosing. There are two domestic PopPK studies[25,31] on the effects of co-administration of tacrolimus with Wuzhi capsule in Chinese renal transplant recipients. These two studies are limited to the effect of Wuzhi Capsule on tacrolimus clearance. To the best of our knowledge, there is no study on the effect of Wuzhi capsule dose on tacrolimus clearance, and it is unclear whether long-term consumption of Wuzhi capsule dose would also increase the blood concentration of tacrolimus. The aim of our study was to confirm the effect of Wuzhi capsule dose on tacrolimus elimination in Chinese kidney transplant recipients. Herein, we showed that an adult patient with *CYP3A5*3/*3* would require a daily tacrolimus dose of 5 mg without co-treatment with Wuzhi capsule with a median creatinine clearance rate of 45.5 mL·min^{-1} and hematocrit of 29%; the typical CL/F was estimated to be 14.4 L·h^{-1}. Using our model, we identified creatinine clearance rate, hematocrit, Wuzhi capsule dosage, CYP3A5*3 gene polymorphism, and daily tacrolimus dose as significant covariates affecting tacrolimus CL/F in the first 3 months following renal transplantation ($p < 0.001$). As the Ka was fixed, we performed sensitivity analysis to assess the effect of these covariates on the CL/F and Vd/F of tacrolimus. As all sampling points were in the elimination stage, Ka did not affect CL/F and Vd/F ($p > 0.05$).

This study revealed that patients with a lower creatinine clearance rate had a higher CL/F of tacrolimus. However, tacrolimus is not excreted through the kidney, and the relationship between tacrolimus and kidney function remains controversial. Most studies[32] on calcineurin

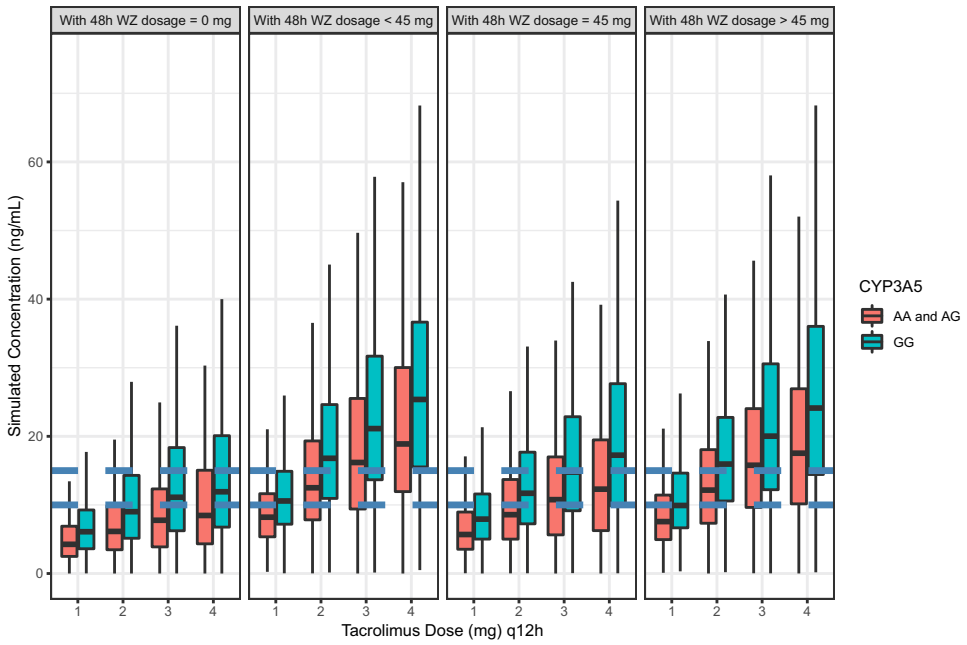

Figure 4 Boxplots of the distributions of simulated tacrolimus trough concentrations for CYP3A5 with AA/AG and GG co-administered Wuzhi capsule on 48h for dosage<45mg,=45mg and >45mg, and group on 1, 2, 3 and 4mg q12h regimens in kidney transplant patients. The bold horizontal bars in the middle show the median values, whereas the outer boundaries of the boxes represent the ranges of the 25th and 75th percentiles (interquartile ranges). The whiskers indicate the maximum and minimum values of trough concentrations.

inhibitor popPK in transplant recipients have not considered renal function as a predictor of dose requirement. However, some studies have reported a significant correlation between serum creatinine level and CL/F.[33,34]

Notably, tacrolimus disposition and renal function relationship may be confused by the intrinsic acute and chronic nephrotoxic effects and the reduced renal function can affect drug disposition,[32] which requires an increase in therapeutic dose. Some studies have reported that if acute changes in renal function occurred, which can affect the absorption and clearance of tacrolimus, renal tubular cells may express the *P-gp* and *CYP3A5* genes.[35,36] Thus, tacrolimus renal metabolism could lead to potentially nephrotoxic tacrolimus metabolite accumulation, which may be influenced by genotypes of *CYP3A5* and *P-gp*.

Haematocrit fraction generally decreases during early post-operative period, and then increases as the kidney functions recover.[37] Tacrolimus binds to erythrocytes (approximately 95%) in a concentration-dependent manner.[32] We observed a negative correlation between tacrolimus CL/F and hematocrit, as a continuous covariate, ie, a decrease in hematocrit resulted in an increased blood concentration of free tacrolimus. In the plasma, tacrolimus binds to proteins, approximately 99% albumin and alpha-1-acid glycoprotein, and is independent of concentration at a range of 5 to 50 ng·mL^{-1}. Clinical studies have confirmed that higher tacrolimus doses are required as hematocrit decreases,[38–42] which is consistent with our current findings.

The present study also confirmed that the *CYP3A5*3* genotype affected tacrolimus CL/F, whereas ABCB1 genotypes did not. We showed that among kidney transplant patients, tacrolimus CL/F of *CYP3A5*1* carriers was 1.29-fold higher than that in non-carriers (*CYP3A5*3/*3*). Enzymes of the CYP3A family metabolize tacrolimus,[43] and CYP3A5 genetic variations constitute 40% to 50% of the variability in tacrolimus systemic clearance.[44] Blood tacrolimus concentrations have been reported to be strongly affected by *CYP3A5* genotypes, and tacrolimus CL/F of *CYP3A5* (*1*1 and *1*3) expressers was higher ($p < 0.001$) than that of *CYP3A5* (*3*3) non-expressers, with a tacrolimus CL/F change of 1.45 (range 1.15–2.5) in CYP3A5 expressers.[45] Clinical Pharmacogenetics Implementation Consortium Guidelines and Second Consensus Report of tacrolimus suggest that a *CYP3A5* expresser would require a higher starting dose than the recommended dose (ie, starting dose is increased by 1.5–2 times) and a CYP3A5 non-expresser would require the standard recommended starting dose. Our finding further confirmed these results concerning the importance of *CYP3A5* genotypes in tacrolimus metabolism. Hence, we

recommend individualizing the initial tacrolimus treatment according to the *CYP3A5* genotype.

Except for the above mentioned factors, drug–drug interactions between tacrolimus and Wuzhi capsule have been widely investigated. Wuzhi capsule considerably increased the blood concentrations of tacrolimus in patients and rats, and the action mechanism has been studied in rats.[46–49] Pharmacokinetic interactions between Wuzhi capsule and tacrolimus have also been studied in rats.[50] Following the administration of Wuzhi capsule at doses of 0, 25, 100, 150, 450, 1000, or 1250 mg/kg in rats, as Wuzhi capsule dosage was increased at a range of 0–450 mg/kg, the mean plasma concentration of tacrolimus increased simultaneously, with the maximum concentration of tacrolimus at 450 mg/kg. When Wuzhi capsule was administered at doses above 450 mg/kg, the tacrolimus $AUC_{0\rightarrow t}$ failed to further increase, whereas the highest Wuzhi dose (1250 mg/kg) did not correspond to the highest effect, which suggested that Wuzhi capsule has a biphasic effect in regulating the pharmacokinetic parameters of tacrolimus. In other words, Wuzhi capsule treatment at 450 mg/kg exerts a stronger inhibitory effect than inductive effect on tacrolimus disposition in rats.

Wuzhi capsule inhibited P-gp-mediated efflux and *CYP3A*-mediated metabolism of tacrolimus, and reduced intestinal first-pass metabolism of tacrolimus was the major cause of increased tacrolimus oral bioavailability by Wuzhi capsule.[47] Long-term pre-treatment with Wuzhi capsule through activation of the orphan nuclear receptor pregnane X receptor induced both *CYP3A* and *CYP2C* expression, which consisted of short-term inhibition followed by long-term induction, on the regulation of *CYP3A* expression. This phenomenon was also discovered in St. John's wort.[48] In rat and human liver microsomes, Wuzhi capsule extract at 100 μM almost completely inhibited tacrolimus metabolism,[48] further supporting that Wuzhi capsule extract potentially inhibited the CYP3A-mediated metabolism of tacrolimus. Consistent with previous study findings, the present study suggested that Wuzhi capsule extract exhibited a concentration- and time-dependent inhibitory effect on *CYP3A*.

In the present study, patients treated with Wuzhi capsule at an accumulated dose of below 45 mg had the lowest tacrolimus clearance rate with a CL/F value of 0.566 $L{\cdot}h^{-1}$, those treated at an accumulated dose of 45 mg had a CL/F value of 0.783 $L{\cdot}h^{-1}$, and those treated at an accumulated dose of greater than 45 mg had a CL/F value of 0.598 $L{\cdot}h^{-1}$. These findings are similar to those of the above studies, which indicated that Wuzhi capsules at lower doses showed greater effect on tacrolimus clearance.

After the inclusion of daily dose of tacrolimus, the effect on CL/F increased with a decrease in the OFV by −138.106 points, which supported the non-linearity of tacrolimus pharmacokinetics. The CL/F of tacrolimus increased non-linearly as the daily dose increased, which is consistent with previous findings in kidney and lung transplant recipients.[8,51,52] This phenomenon can be explained by the poor water solubility of tacrolimus and its low transmembrane permeability in the intestine, which may result in its low and variable oral bioavailability and limited absorption rate in the gastrointestinal tract.[2] The non-linear kinetics of tacrolimus warrant further investigation with a larger sample size.

The adjustment of tacrolimus dose in the co-treatment with Wuzhi capsule remains an unmet need. Model simulation is used to design different clinical scenarios to simulate the concentration. By analyzing different combinations of tacrolimus doses and treatment with or without Wuzhi capsule, we provided a tacrolimus dose recommendation for co-treatment with Wuzhi capsule. According to the Chinese Renal Transplant Recipients Association guidelines for immunosuppressive therapy, the reference value of target trough tacrolimus concentration within 1 month of operation is 10–15 $ng{\cdot}mL^{-1}$ in a triple therapy with tacrolimus + MPA drugs + hormones. Our simulated values will be compared with the reference values.

There were some limitations to this study. First, we were unable to elucidate the effect of Wuzhi capsule on the absorption and distribution of tacrolimus owing to the limited samples. To date, although pharmacodynamic and immune-related biomarkers have been recommended by the guidelines, these have not been included in routine monitoring. We will obtain this information to build other popPK models in the future.

Second, due to the small sample size, we could not combine different genotypes of CYP3A5 with different dose groups of Wuzhi capsule. We will expand the sample size in future studies. Furthermore, data from intravenous administration were not obtained in this study, even though absorption should be investigated along with distribution and elimination. Therefore, we only estimated CL/F and Vd/F. However, despite these limitations, the current study provides a fundamental theory for individualized tacrolimus treatment when combined with Wuzhi capsules and served as a valuable model for adult kidney transplant. Additional

analyses are required to evaluate the potential effects of these covariates, especially Wuzhi capsules, in the clinical implementation of these models to guide tacrolimus dosing.

Conclusion

In summary, a popPK model was established to characterize the pharmacokinetics of tacrolimus in Chinese kidney transplant recipients. Significant covariates were identified in the final model, including creatinine clearance rate, haematocrit, dosage of Wuzhi capsule, and *CYP3A5*3* gene polymorphisms. To the best of our knowledge, this is the first popPK study to reveal the dose-dependent interactions between Wuzhi capsules and tacrolimus pharmacokinetics. Our findings showed that low-dose Wuzhi capsule exerted the optimum effect on tacrolimus pharmacokinetics. However, to elucidate the specific mechanisms underlying this effect, further studies need to be conducted Our results revealed that in a combination treatment with tacrolimus and Wuzhi capsule, popPK modeling and TDM can be implemented to optimize the dosing regimen and maintain trough tacrolimus concentration, which could ensure the efficacy and safety of tacrolimus.

Data Sharing Statement

The raw data required to reproduce these findings cannot be shared at this time as the data also form part of an ongoing study.

Informed Consent

Written informed consents were obtained from all individual participants included in the study. The organ donors provided informed consent to donate their kidneys.

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Disclosure

The authors report no conflicts of interest in this work.

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