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ORIGINAL ARTICLE

Population pharmacokinetics of tacrolimus in children with nephrotic syndrome

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AIMS

Nephrotic syndrome (NS) is the most common clinical manifestation of glomerular disease in children. Currently, tacrolimus (TAC) is widely used in children with NS. However, pharmacokinetic data in children with nephrotic syndrome is limited. This study was intended to evaluate the population pharmacokinetics (PPK) of TAC in paediatric NS and to optimize dosing regimen.

METHODS

Blood samples from NS children treated with TAC were collected and the blood concentrations of TAC were detected using HPLC-MS/MS. A PPK model was developed using NONMEM software. Pharmacogenetic analysis was carried out in the CYP3A5 gene.

RESULTS

The data from 28 children were used for PPK analysis. A one-compartment model and first-order elimination were accorded with the TAC data in paediatric NS. A covariate analysis showed that body weight and CYP3A5 genotype significantly affected TAC pharmacokinetics. Monte Carlo simulation indicated that NS children with CYP3A5*3/*3 receiving 0.10 mg kg^{-1} $dose^{-1}$ twice daily and NS children with CYP3A5*1 receiving 0.25 mg kg^{-1} $dose^{-1}$ twice daily TAC could achieve the target concentrations of 5–10 ng ml^{-1}.

CONCLUSION

The PPK of TAC was estimated in children with NS and a CYP3A5 genotype-based dosing regimen was set up based on simulations.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Tacrolimus is used in the treatment of children with nephrotic syndrome.
- CYP3A5 gene polymorphism significantly affected tacrolimus pharmacokinetics and clinical outcomes in solid organ transplantation patients.

WHAT THIS STUDY ADDS

- Body weight and CYP3A5 genotype had significant impact on tacrolimus pharmacokinetics in children with nephrotic syndrome.
- A CYP3A5 genotype-based dosing regimen in children with nephrotic syndrome was formulated.

Introduction

Nephrotic syndrome (NS) is the most common glomerular disease in childhood with clinical manifestation of proteinuria, hypoalbuminaemia, hyperlipidaemia, and oedema [1, 2]. Minimal change nephropathy (MCN) is the most common pathological type of NS in paediatrics. Although more than 90% of children with MCN were reported to achieve remission with oral corticosteroid therapy, recurrence is common, bringing about an increase in morbidity, complications, treatment costs and a decline in quality of life [3]. In addition, the majority of children with focal segmental glomerulosclerosis (FSGS), the second most common pathological type, do not respond to corticosteroids [4].

Tacrolimus (TAC), a calcineurin inhibitor, was labelled to prevent or treat rejection in organ transplant patients. In clinical practice, TAC is also commonly used for the treatment of nephrotic syndrome in an off-label manner. The first published reports can be found as early as the 1990s in adults and 2000s in paediatric patients [5–8].

Several studies suggest that the immune system plays a vital role in NS [9, 10]. TAC inhibits the activation of a necessary transcription factor in T cells, which is indispensable for the transcription of cytokine genes, resulting in a decreased production of cytokines such as IFN-γ and IL-2 [11, 12]. Recently, it is believed that podocyte injury is the central event of nephrotic syndrome [13]. Studies have shown that TAC can inhibit the specific phosphatase activity of podocyte, stabilize the actin skeleton of podocyte, and reduce proteinuria [14, 15].

Although the efficacy and safety data are available in children with NS, the clinical application of TAC remains hampered by its narrow therapeutic index (TI) and high inter- and intra-individual pharmacokinetic variability [16, 17]. Both under- and overexposure to TAC may have serious consequences, increasing the risk of treatment failure or toxic side effects [18].

TAC is extensively metabolized in the liver and undergoes biliary excretion. **CYP3A5** is the predominant enzyme for metabolism of TAC [19, 20]. CYP3A5 gene polymorphism significantly affects TAC pharmacokinetics and clinical outcomes in solid organ transplantation patients [21–26]. Although the pharmacokinetics of TAC have been evaluated in paediatric organ transplant patients, these data cannot be directly used in NS patients because the organ transplant patients had hypohepatia and/or rapid change of renal function after transplant and the liver enzyme activity showed a modest derangement during TAC treatment [27]. It has been reported that time post-transplantation in days, hematocrit, liver weight and liver function influenced tacrolimus elimination [28–31]. Nephrotic syndrome induces proteinuria, hypoalbuminemia, hypertriglyceridemia and hypercholesterolemia [32, 33]. These characteristics may have an impact on TAC pharmacokinetics. Gut oedema secondary to hypoproteinaemia may affect drug absorption, while hypoalbuminaemia can reduce protein binding of the drug and thus alter volume of distribution (V) or clearance (CL), as TAC is highly bound to plasma proteins [34]. All these clinical features in organ transplant patients lead to their unique pharmacokinetic characteristics, which cannot be directly extrapolated to children with NS [35].

Given the limited available pharmacokinetic data in paediatric patients with NS and unmet need in clinical practice to optimize TAC therapy, we conducted this study aiming (i) to develop a PPK model of TAC in children with NS; (ii) to evaluate impacts of CYP3A5 genotype as well as patient characteristics on TAC pharmacokinetic parameters; and (iii) to optimize TAC dosing regimens based on the developed model.

Methods

Study design

This pharmacokinetic study was a prospective, open label trial, carried out in our department of paediatric nephology from 2015 to 2017 in the Children's hospital of Hebei province, China. The inclusion criteria included: children under 18 years of age with NS; treated with TAC as initial immunosuppressant. The exclusion criteria included: children with a concomitant medical condition, whose participation, according to the opinion of the Investigators, may lead to unacceptable additional risks. The study, designed in accordance with requirements of the law and the Declaration of Helsinki, was approved by the institutional ethics board, and registered at ClinicalTrials.gov (ID number NCT03347357). Informed consent was signed by the children's parents or guardians.

Dosing regimen and pharmacokinetic sampling

Tacrolimus (Prograf, Astellas, Japan), was administered orally at a dose of 0.05 mg kg^{-1} $dose^{-1}$ twice daily. A full concentration–time profile was confirmed when a steady-state condition was achieved during hospitalization. Blood samples were extracted before and at 1, 2, 3, 6, 9 and 12 h after taking TAC. Precise medication times and sampling times

were recorded. Each blood sample used for pharmacokinetic analysis had a volume of 0.2 ml.

Analytical method of tacrolimus and genotyping

Tacrolimus blood concentrations were determined by high-performance liquid chromatography with tandem mass spectrometry. The range of calibration curve was from 2.0 to 100 ng ml^{-1}, and the lower limit of quantification (LOQ) was 2.0 ng ml^{-1}. The intraday and interday coefficients of variation were 4.4 and 7.2%, respectively.

Total genomic DNA was extracted from blood samples using a TIANamp Blood Clot DNA Kit (TIANGEN Biotech, Beijing, China) according to the manufacturer's protocol. CYP3A5 A6986G (rs776746) polymorphism was determined using the TaqMan (Thermo Fisher Scientific, MA, USA) allelic discrimination technique with 3′-minor groove binding quencher probes on a Bio-Rad Fluorescence Quantitative PCR (Hercules, CA, USA) according to manufacturer's instructions.

Population pharmacokinetic modelling of tacrolimus

The PPK analysis was conducted by the nonlinear mixed-effects modelling program NONMEM (Version 7.2, Icon Development Solutions, USA). The first-order conditional estimation (FOCE) with interaction method was selected throughout the model-building procedure to estimate the pharmacokinetic (PK) parameters and their variability.

An exponential model was used to estimate interindividual variability of the PK parameters and could be expressed as follows:

$$\theta_i = \theta_{mean}{}^{*} \exp(\eta_i)$$

where θ_{mean} is the typical population value of the parameter, θ_i is the individual pharmacokinetic parameter value of the ith subject, and η_i is the difference between the ith individuals' θ and the predicted values. The values of η_i are random, independent, identically distributed variables, following the normal distribution with mean 0 and variance ω^2.

The selection of covariates followed a forward-selection process and a backward-elimination process. The effect of each variable on parameters was tested using likelihood ratio. Body weight, age and CYP3A5 genotype were investigated as potential variables affecting PK parameters. In the forward-selection process, a covariate was added to the model if the objective function value (OFV) significantly ($P < 0.05$) decreased (reduction>3.84) from the basic model. All the statistically significant covariates were included in the full model. Then, in order to re-evaluate the importance of these variables, each was independently removed from the full model. The covariate was retained in the final model only if the increase (increase > 3.84) of OFV was significant ($P < 0.05$).

The final model was validated based on statistical and graphical criteria. Goodness-of-fit was evaluated using diagnostic scatter plots, including: (i) observed (DV) *vs*. population predicted concentrations (PRED); (ii) DV *vs*. individual predicted concentrations (IPRED); (iii) conditional weighted residuals (CWRES) *vs*. time; (iv) CWRES *vs*. PRED. The stability of the final model was evaluated by the nonparametric bootstrap with re-sampling and replacement. Re-sampling was repeated 1000 times. The values of estimated parameters from the bootstrap procedure, such as the medians and SEs, were compared with those estimated from the original dataset. The final model was also evaluated using normalized prediction distribution errors (NPDE) [36, 37]. The dataset was simulated 1000 times using the final model parameters. The NPDE is expected to follow the $N(0, 1)$ distribution. NPDE results were summarized graphically and the following graphs were plotted by using the NPDE R package (version 1.2) [38]: (i) QQ-plot of the NPDE; (ii) histogram of the NPDE.

Dosing regimen optimization

Monte Carlo simulations were carried out using the parameter estimates from the final population model [39, 40]. The aim was to determine an optimal dosing regimen and achieve the target trough concentration (C_0) of 5–10 ng ml^{-1} [41]. The dose of TAC was simulated on a 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 mg kg^{-1} $dose^{-1}$ twice daily basis according to different CYP3A5 genotype groups. One thousand simulations were carried out using the initial dataset, and steady-state C_0 of each simulated subject was calculated. An optimal dosing regimen of tacrolimus was then established based on the median of simulated C_0 in each CYP3A5 genotype group.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [42], and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 [43].

Results

Study population

A total of 28 patients were included in final pharmacokinetic analysis. All the participants met the inclusion criteria and signed informed consent. The mean (SD) age and body weight of the 28 participants were 9.5 (4.4) (range 2.7–17.3) years and 36.5 (17.4) (range 12.9–81.0) kg, respectively. The patient features are listed in Table 1.

Model building

For PPK modelling, a total of 148 TAC concentrations were obtained from 28 children. The TAC concentrations ranged from <2.0 (n = 11) to 38.7 ng ml^{-1}. Half of the LOQ value was used to handle concentrations below the LOQ in PK modelling. The curve of concentration varying with time is shown in Figure 1.

A one-compartment model and first-order elimination were more suitable for describing TAC concentrations in paediatric NS. The proportional model best described residual variability. The model was parameterized in terms of clearance (CL), volume of distribution (V) and absorption rate constant (k_a) of TAC. The inter-individual variability (IIV)

Table 1

Characteristics of the 28 paediatric nephrotic syndrome patients

Patient characteristic	Number	Mean	SD	Median	Range
No. of patients	28				
Male/female	19/9				
Age (year)		9.5	4.4	9.4	2.7–17.3
Body weight (kg)		36.5	17.4	30.0	12.9–81.0
Tacrolimus dose (mg, twice daily)		3.8	2.2	4.0	1.0–8.0
Tacrolimus dose (mg kg^{-1}, twice daily)		0.1199	0.0860	0.0909	0.0222–0.3876
Pharmacokinetic data					
Samples (*n*)	148				
Concentrations (ng ml^{-1})					<2–38.7
Samples per patient					1–7
CYP3A5					
***3/*3**	21				
***1/*3**	6				
***1/*1**	1				

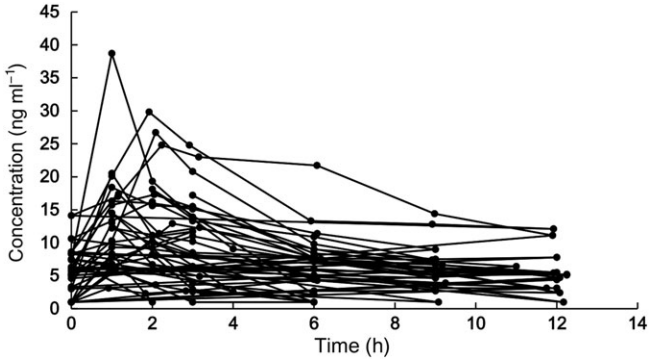

Figure 1

The concentration *vs.* time profile

was modelled using the exponential error model and was then estimated for CL, V and k_a.

Covariate analysis

The allometric size approach was used by implementing the body weight into the basic model (the allometric coefficients fixed at 0.75 for CL and 1 for V). After implementing body weight, OFV significantly decreased by 5.462 units. A further drop in the OFV of 4.961 points occurred when CYP3A5 genotype was incorporated on CL. Parameter estimates of the final PK model are shown in Table 2. The median (range) of estimated CL and V at steady state were 0.595 (0.211–1.933) l h^{-1} kg^{-1} and 4.688 (0.924–39.389) l kg^{-1}, respectively. CL of TAC in children with NS increased allometrically with body weight.

$$\mathrm{CL/F} = \theta 2 \times (\text{body weight}/70)^{0.75} \times \theta 3^{\mathrm{FLAG1}}$$

where FLAG1 = 1 for the CYP3A5*1 allele; FLAG1 = 0 for CYP3A5*3/*3.

CL/F was significantly lower in children with CYP3A5*3/*3 compared with children with the CYP3A5*1 allele (0.567 ± 0.216 *vs.* 1.050 ± 0.641 l h^{-1} kg^{-1}, $P = 0.005$).

Model validation

Acceptable goodness-of-fit for the final model of TAC was shown by model diagnostics. Figures 2A and 2B show that predictions are unbiased. No trends were observed (Figures 2C and 2D) in the diagnostic plots of CWRES *vs.* time and PRED. Furthermore, as shown in Table 2, the median parameter estimates obtained by the bootstrap process are consistent with the respective values of the final model, demonstrating that the final population model is stable, and it can reconfirm the estimated value of the PPK parameters.

The NPDE results are shown in Figure 3. The mean of NPDE was 0.0185 (Wilcoxon signed rank test $P = 0.902$) and the variance was 0.871 (Fisher variance test = 0.263). The NPDE distribution and histogram accorded with the theoretical $N(0, 1)$ distribution and density, showing that the model fitted the individual data.

Dosing regimen optimization

Monte Carlo simulation showed that patients with CYP3A5*3/*3 receiving tacrolimus 0.10 mg kg^{-1} twice daily reached a median steady state C_0 of 8.1 ng ml^{-1} and patients with CYP3A5*1 receiving 0.25 mg kg^{-1} twice daily had a median C_0 of 7.6 ng ml^{-1}. The simulation results are presented in Figure 4.

Table 2

Population pharmacokinetic parameters of tacrolimus and bootstrap validation (n = 1000)

	PK parameters	SE (%)	Bootstrap		
			Median	5th	95th
Absorption rate constant (h^{-1}) k_a	5.21	17.1	5.23	2.68	7.08
Volume of distribution (L) V/F					
V/F = θ1 × (bodyweight /70)					
θ1	411	20.9	413	296	609
Oral clearance ($l\ h^{-1}$) CL/F					
CL/F = θ2 × (bodyweight/70)$^{0.75}$ × F_{CYP3A5}					
θ2	30.9	9.2	30.9	26.3	35.8
If CYP3A5 *1/*1, F_{CYP3A5} = θ3					
If CYP3A5 *1/*3 F_{CYP3A5} = θ3					
If CYP3A5 *3/*3, F_{CYP3A5} = 1					
θ3	1.60	23.8	1.64	1.11	2.42
Inter-individual variability (%)					
k_a	79.1	52.8	75.1	38.9	142.5
V/F	99.4	34.3	96.7	70.4	126.7
CL/F	43.8	33.0	40.6	29.2	52.3
Residual variability (exponential) (%)	25.9	22.2	25.6	21.2	30.7

Discussion

Population pharmacokinetic study of TAC was first conducted in children with NS. The purpose of this study was to assess the PK parameters of TAC and to assess the effects of biological, demographic and clinical factors on TAC disposition. A one-compartment model with first-order elimination with body weight and CYP3A5 genotype as covariates was established.

In the present model, the mean CL/F of TAC is 0.69 $l\ h^{-1}\ kg^{-1}$, which is consistent with the value in kidney transplant children (0.76 $l\ h^{-1}\ kg^{-1}$) [44]. However, the half-life in paediatric nephrotic syndrome patients in our study (about 9 h) seems to be much shorter than observed in patients that have undergone renal transplantation (about 24 h). TAC is highly bound to plasma proteins. Nephrotic syndrome may cause severe hypoproteinaemia. The percentage of unbound TAC in the NS patients will be higher than that in renal transplantation patients [45]. Therefore, patients with nephrotic syndrome have lower apparent distribution volume and a greater elimination rate constant (k_e) than those with renal transplantation.

After forward-selection and backward-elimination processes, body weight and CYP3A5 gene polymorphisms were identified as significant covariates associated with interindividual variability. An allometry-based PPK model was developed for TAC: body weight was a biological factor with allometric coefficients of 0.75 for CL and 1 for V, respectively. These quarter-power models have observational and theoretical bases in biology [46, 47]. The same model was used for children with kidney transplant [48, 49].

As CYP3A5 plays a vital role in TAC metabolism, a possible association was investigated between CYP3A5 polymorphism and TAC CL/F. It has been reported that kidney and liver microsomes from donors with a CYP3A5*1/*3 genotype had a higher TAC clearance than those with CYP3A5*3/*3 genotype [19]. Our results demonstrated that the weight normalized CL/F of tacrolimus was significantly higher in expressers (CYP3A5*1 allele) than in non-expressers (CYP3A5*3/*3). CL/F in CYP3A5*1 carriers was 1.6-fold higher than in CYP3A5*3/*3 carriers (non-expressers) in our study. Similarly, the ratio was 1.66, 1.8 and 1.45 in paediatric and adolescent kidney transplant recipients [48–50].

Tacrolimus has gained acceptance in the treatment of NS in children [7, 51–53]. The recommended target C_0 was 5–10 ng ml^{-1} in children with NS [8, 54], which was lower than 10–20 ng ml^{-1} in the immediate post-transplantation period used for all types of paediatric organ transplantations [55]. There is growing evidence to support a potential benefit for CYP3A5 genotyping before the initiation of tacrolimus-based immunosuppressive therapy in children undergoing organ transplantation [56, 57]. We have proved that a standard TAC dosage of 0.10 mg kg^{-1} $dose^{-1}$ twice daily may result in underexposure to TAC in NS children with the CYP3A5*1 allele, and that the higher dosage (0.25 mg kg^{-1} $dose^{-1}$ twice daily) should be suggested.

There are some limitations in our study. Due to the limited number of participants, the PPK model of TAC was established and internally validated only. External validation will be carried out after applying it in clinical practice. Randomized controlled studies are definitely required prior to implementing a personalized approach as a 'standard of care'.

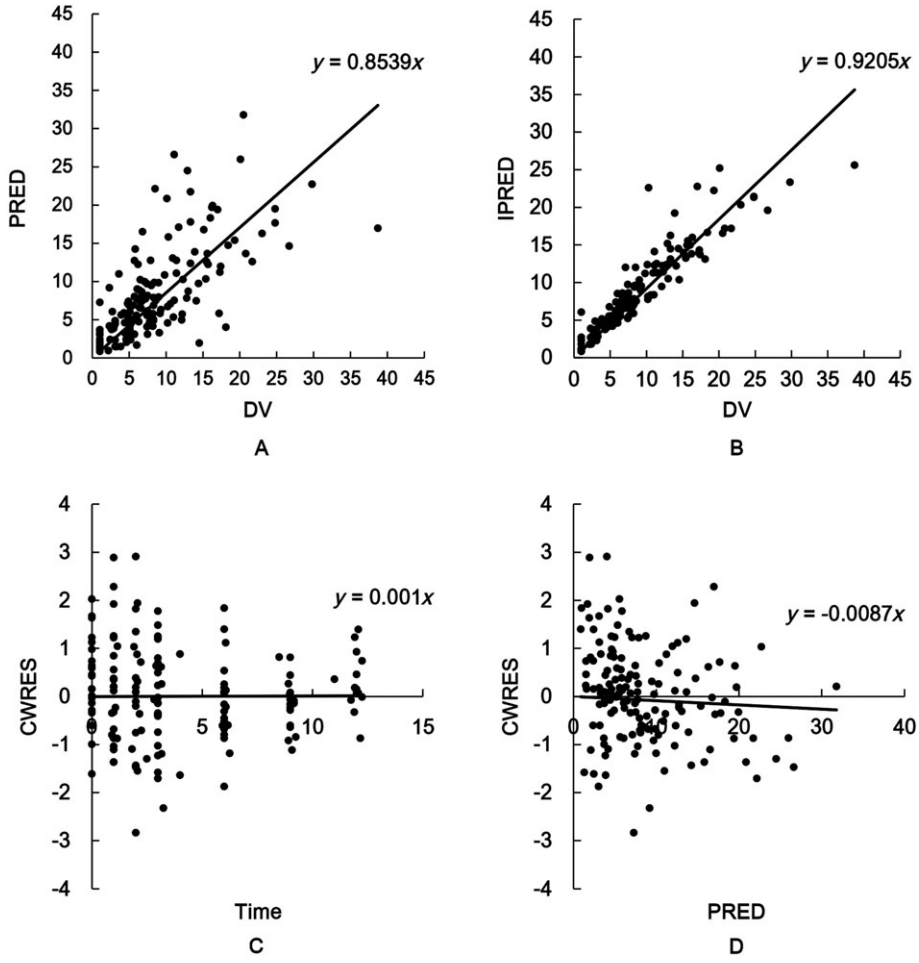

Figure 2

Diagnostic goodness-of-fit plots for the final population pharmacokinetic model of tacrolimus, including (A) observed (DV) *vs*. population prediction (PRED); (B) DV *vs*. individual prediction (IPRED); (C) time *vs*. conditional weighted residuals (CWRES); and (D) PRED *vs*. CWRES

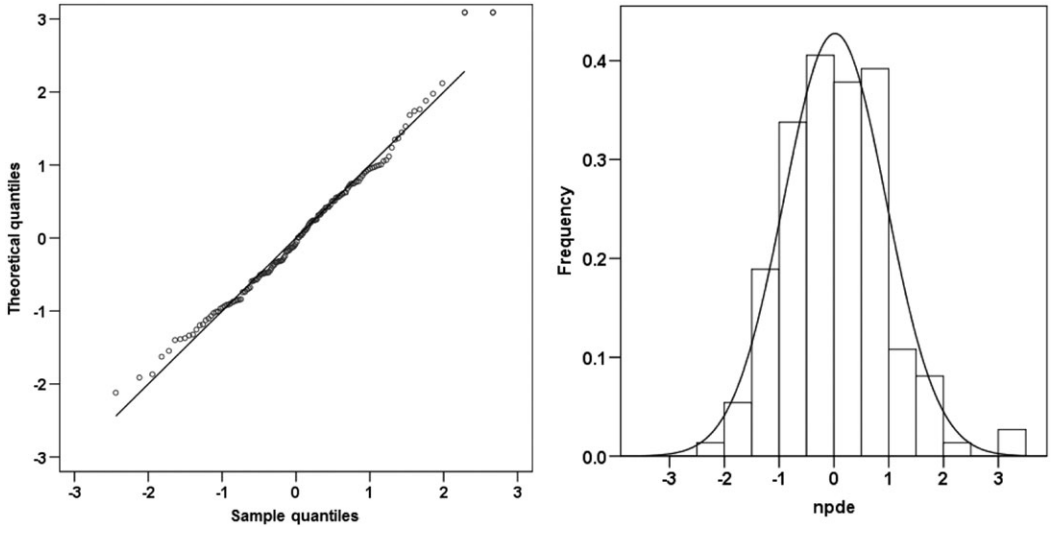

Figure 3

Normalized prediction distribution errors (NPDE) analysis for the tacrolimus final model. NPDE: QQ-plot of the distribution of the NPDE versus the theoretical *N*(0,1) distribution (left). Histogram of the distribution of the NPDE, with the density of the standard Gaussian distribution overlaid (right)

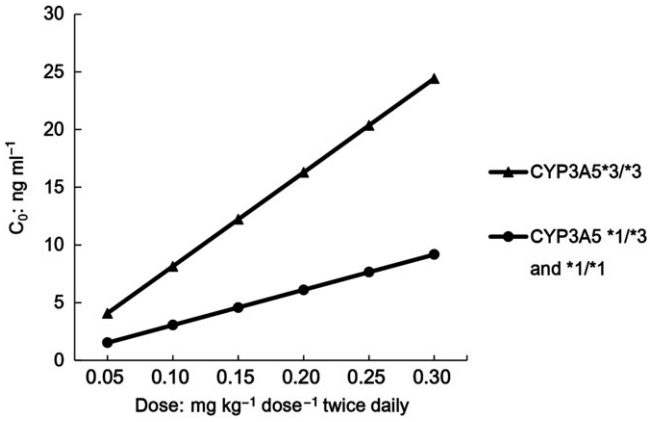

Figure 4
Simulation of the median for tacrolimus steady state C_0 obtained in patients of two genetic backgrounds

Ultimately, the CYP3A5 genotype-based dose regimen must be applied in clinical practice to identify its benefits in children with NS.

Conclusion

The PPK model of tacrolimus was established in children with nephrotic syndrome. Body weight and CYP3A5 genotype significantly affected pharmacokinetics of tacrolimus. A CYP3A5 genotype-based dosing regimen was developed based on the PPK analysis.

Competing Interest

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author). None of the authors have any other relationships or activities that could appear to have influenced the submitted work. This work is supported by the National Natural Science Foundation of China (81503163), National Science and Technology Major Projects for Major New Drugs Innovation and Development (2017ZX09304029-002) and Young Taishan Scholars Program and Young Scholars Program of Shandong University.

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