Original Article
The population pharmacokinetics of sirolimus and CYP3A5*3 polymorphism in Chinese renal transplant patients

Hao-Qiang Shi1*, Jun Yang2*, Li-Qun Zhang1, Bei-Ming Xu1, Hui-Lan Lu2, Er-Zhen Chen3, Bing Chen1

Departments of 1Pharmacy, 3Intensive Care Unit, Ruijin Hospital, Shanghai Jiaotong University, School of Medicine, Shanghai 200025, China; 2Department of Pharmacy, Shanghai Xiang-Shan Hospital, Shanghai 200025, China. *Equal contributors and co-first authors.

Received November 8, 2015; Accepted February 10, 2016; Epub March 15, 2016; Published March 30, 2016

Abstract: Objectives: Sirolimus (SRL) is a widely used immunosuppressive agent in preventing allograft rejection after solid organ transplantation. In this study we established a population pharmacokinetic (PPK) model for SRL in Chinese renal transplant patients, and elucidated the influence of CYP3A5*3 genotypes on SRL PPK parameters and SRL dosing regimen in Chinese renal transplant recipients. Methods: 108 renal transplant patients were enrolled retrospectively. The trough concentration (C₀) of SRL in steady state was monitored and pathophysiological data were recorded. The CYP3A5*3 genotypes was determined for each patients. The NONMEM software was used to establish the PPK model for SRL. The influence of age, gender, body weight (BW), renal and liver function, CYP3A5*3 genotype on PPK parameters was evaluated. Results: 915 C₀ were obtained from 108 patients. The average C₀ was 6.09 ± 3.27 ng/ml. There were 11, 36, 61 patients with CYP3A5*1/*1, *1/*3 and *3/*3 genotype. Single compartment model was the most suitable model in Chinese renal transplant patients. The CL/F, Vd/F and Ka were 10.9 ± 0.99 L/h, 357 ± 102 L and 2.20 l/h. BW (P<0.01), albumin level (P<0.01) and CYP3A5*3 genotype (P<0.01) were found to have significant influence on CL/F of SRL. By using Bayesian method, dosage of SRL to reach the target concentration for CYP3A5*1/*1, *1/*3 and *3/*3 patients were predicted as 2.23:1.77:1. Conclusion: The PPK model established can be used to estimate individualized SRL pharmacokinetic parameters. On the basis of TDM data of SRL, patients pathophysiological data and CYP3A5 genotype, the initial and maintain dosage of SRL in Chinese renal transplant patient can be simulated and individualized immunosuppressive regimen can be designed.

Keywords: Sirolimus (SRL), CYP3A5, genotype, population pharmacokinetics (PPK), NONMEM

Introduction
Sirolimus (SRL) is a macrocyclic lactone which is widely used as an immunosuppressive agent in preventing allograft rejection after solid organ transplantation [1, 2]. The mechanism of SRL immunosuppressive effect is through binding with FK506 binding protein 12 (FKBP-12) to form a complex that inhibits mammalian target of rapamycin (mTOR), thereby suppressing T lymphocyte proliferation [3-6]. After administration, SRL is rapidly absorbed from the gastrointestinal tract with the bioavailability of about 15% in patients in steady state [7, 8]. Previous study proved that terminal phase half-life of SRL is as long as about 60 h in kidney transplant recipients [9]. There is great inter-individual variety in the CL/F of SRL, the mean values of CL/F ranged from 7.1 to 28 L/h in healthy subjects and liver or renal transplant patients [10-15]. Physiological and pathological factors of patients were reported to have impact on the pharmacokinetics (PK) of SRL. SRL is metabolized by Cytochrome P450 3A in vivo. CYP3A4 and CYP3A5 located in gut mucosa and liver are responsible for the demethylation and hydroxylation of SRL. More than 5 different SRL metabolites have been detected, including hydroxyl, dihydroxy, trihydroxy, desmethyl and didesmethyl SRL. All metabolites have low activity of immunosuppressive effect. It was found demethyl and hydroxy SRL had
Population pharmacokinetics of sirolimus

immunosuppressive activity about 7% and 10% of parent drug [16]. The importance of genetic polymorphism of CYP3A4 or CYP3A5 on PK of SRL was also suggested. CYP3A5 represents at least half of the total hepatic CYP3A content in persons expressing CYP3A5 and play very important role in the metabolism of SRL. The polymorphic CYP3A5 activity in the human liver and small intestine is strongly dependent on the presence of the CYP3A5*3 allele [19, 20]. The influence of CYP3A5*3 genotype on SRL PK has been proved by previous studies. On the other hand, the impact of CYP3A4 alleles (*4, *5, *6 and *19) is limited for their low frequency (<2% in Chinese subjects) [17, 18].

The individualized dosage is valuable for patients receiving SRL therapy. The exposure of SRL has good correlation with immunosuppressive therapy outcomes such as acute rejection episodes and chronic rejection. Therapeutic drug monitoring (TDM) is conducted routinely and may provide a useful tool in the dosing regimen regulation [21-24]. Trough concentration (C₀) of SRL at steady state was considered to correlate well with AUC and is the most widely used therapeutic index of SRL. The dosage of SRL should be regulated to guarantee the C₀ in the range of 5-9 ng/ml for various immunosuppressive therapy regimens. Due to the long elimination half-life of SRL, TDM should be carried on long after the SRL therapy. Initial dosage estimation on the basis of patient’s pathophysiological condition is helpful to reach the therapeutic target quickly. Pharmacokinetic parameters include CL/F and Vd/F is useful in the design and regulation of SRL dosing regimen for a specific patient. Population methods are particularly suitable in modeling pharmacokinetic responses in a relatively large group of subjects in which there are only limited observations for each subject. More importantly population pharmacokinetic (PPK) has additional benefit of being able to provide quantitative estimates of the interpatient variability of pharmacokinetic response, the intrapatient variability, and the influence of demographic, clinical and genetic factors on the pharmacokinetics. Individualized PPK including CL/F can be estimated by Bayesian method on the basis of a PPK model. Most of SRL PPK studies were carried on in renal transplant patients [14, 15, 25-27]. Other studies included heart transplant patients [14], neurofibromatosis patients [28] and cancer patients [29]. The PPK dataset of some studies on SRL include full pharmacokinetic profiles (full-PK) [15, 25], and some other studies used conventional TDM data including C₀ to establish SRL PPK model [26, 27]. Although the role of genetic polymorphism on the disposition of SRL has been confirmed, most of PPK studies did not evaluate the influence of CYP3A5 polymorphism on SRL pharmacokinetics. In this study, we investigated PPK parameters in Chinese adult patients who were administered oral SRL for immunosuppressive therapy after renal transplantation. We also elucidated the influence of various factors including CYP3A5*3 genotypes on SRL PPK parameters and SRL dosing regimen in Chinese renal transplant recipients.

Methods

Patients

108 (79 males and 29 females) renal transplant recipients were enrolled. All patients were Han nationality. The study was approved by the Ethics Committee of Shanghai Ruijin hospital. Informed consents were obtained from all patients. Different immunosuppressive regimen was selected: (1) SRL was given as part of the primary immunosuppressive regimen in combined with mycophenolate mofetil (MMF), dosage of MMF was 500-1000 mg, q12h; (2) SRL was given in combined with a reduced dosage of cyclosporine (CsA) or tacrolimus (TAC), dosage of CsA and TAC was 100-300 mg·d⁻¹ and 1-5 mg·d⁻¹, respectively; (3) SRL was administered to replace CsA or TAC during therapy. The loading dose of SRL (Rapamune®, 1 mg per tablet; Wyeth Co., Madison, NJ, USA) was usually 6 mg, and the maintenance dose was 2 mg qd. The dosage was further adjusted according to blood level of SRL. According to previous study in Chinese patients [30] and our experience, the SRL level should be maintained in the range of 5-9 ng/ml.

The demographic data including: body weight (BW), age, gender; biological and clinical data including albumin (ALB), total bilirubin (TBIL), serum creatinine, alanine aminotransferase, and aspartate aminotransferase were recorded on the day of SRL monitoring. Clearance of creatinine (CLcr) of the patients was calculated according to Cockcroft-Gault formula [31, 32]. Post operation date (POD) was expressed as the days between operation date and TDM date. Patients taking medication known to affect SRL blood levels were also recorded. To guaran-
Determination of SRL concentrations in whole blood samples

The trough blood (C₀) samples of SRL were drawn at 8:00 a.m., just prior to the administration of SRL. All samples were anticogulated with ethylene diamine tetraacetic acid (EDTA). Whole blood SRL level was measured using a Micro-partical enzyme immune assay (MEIA) on an ARCHITECT i4000 System (Abbott diagnostic, Chicago, IL, USA) using the sirolimus reagent kit. The functional sensitivity of the assay is 1 ng·mL⁻¹.

Genotyping

Leukocyte DNA was extracted by phenol-chloroform method. A tetra-primers amplification method was used to detect CYP3A5*3 allele [33]. 25 µl reaction system contained 15~50 ng DNA, 0.5 U Heat start Taq DNA polymerase (Bio Basic Co. Ltd, Ontario, Canada), 1 × PCR buffer, 0.2 mmol×L⁻¹ dNTP, 1.5 mmol×L⁻¹ MgCl₂, 0.3 µmol×L⁻¹ of each flanking primers (3A5P1: 5’GCC CTT GCA GCA TTT AGT CCT T3’ and 3A5P2: 5’ CCT GCC TTC AAT TTT TCA CTG 3’) and 0.45 µmol×L⁻¹ of each allele specific primers (3A5wt: 5’ CCA AAC AGG GAA GAG ATA T 3’ and 3A5mu: 5’ GAG CTC TTT TGT CTT TCA G 3’). The reaction was carried out according to following program: 15 min at 94°C; followed by 35 cycles of 94°C for 30 s, 57°C for 60 s and 72°C for 60 s with a final extension at 72°C for 7 min. PCR products were analyzed by electrophoresis with 2% agarose gels.

PPK modeling

One- and two- compartment models were evaluated in model construction. Modeling was performed with NONMEM (Version 6, GloboMax, Hanover, MD). Log-transformed concentration data were used to ensure the random effects are sufficiently distributed around zero. The first order conditional estimation method (FOCE) was applied for the modeling. Model selection was based on the objective function value (OFV), parameter estimates and standard errors. OFV is proportional to -2 log likelihood of the relevant model, and lower value indicates a better model. The distribution of empirical Bayes estimates was also important factors for model selection. The primary pharmacokinetic parameters were clearance (CL/F) and volume of distribution (Vd/F). Different Kₐ was tested based on previously reported literature values [15, 25-27].

Interindividual and residual error model

The inter-individual variability (IIV) of the parameters was assessed using an exponential function: \( P_i = TV(P) \times e^{\eta_i} \). Where \( P_i \) was the individual value, \( TV(P) \) was the population value for the parameters described in the equation, \( \eta_i \) was the random deviation of \( P_i \) from \( TV(P) \). The values of \( \eta_i \) were assumed to be independently normally distributed with mean of 0 and variance of \( \omega^2 \).

As the concentration data were log transformed, an additive model was used for residual error analysis: \( \ln(C_{obs}) = \ln(C_{pred}) + \epsilon \). Where \( C_{obs} \) is the observed concentration, \( C_{pred} \) is the predicted concentration, and \( \epsilon \) is residual error with mean of 0 and variance of \( \sigma^2 \).

Covariates

Patients’ physiological and pathological characteristics were evaluated as the possible covariates of SRL pharmacokinetic model. For categorical covariates such as gender and CYP3A5*3 genotypes, discrete numbers were given to each index: 0 and 1 for male and female patients. 0, 1, 2 for CYP3A5*1/*1, *1/*3 and *3/*3 patients. The influence of different covariates on the pharmacokinetic parameter \( TV(P) \) was modeled according to the following equations:

\[ TV(P) = \theta_p \times (\text{covariate}) \]

\[ TV(P) = \theta_p + q_c \times (\text{covariate}) \]

\[ TV(P) = \theta_p \times e^{(\text{covariate} \times \theta_c)} \]

\[ TV(P) = \theta_p \times (\text{covariate/means of covariate})^{\theta_c} \]

Where \( TV(P) \) is the typical value of the pharmacokinetic parameters, \( \theta_p \) is the population estimation of the parameter, \( \theta_c \) is the factor contributed by the covariate.

A forward inclusion and backward elimination techniques were used for the final regression model. Each candidate covariate was screened in turn by adding into the base model. Weighted
### Table 1. Demographic and Clinical Data of 108 renal transplant recipients with the therapy of sirolimus

<table>
<thead>
<tr>
<th>Characters</th>
<th>CYP3A5*1/*1</th>
<th>CYP3A5*1/*3</th>
<th>CYP3A5*3/*3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age year</strong></td>
<td>44.1 ± 9.9 (28~67)</td>
<td>44.7 ± 12.7 (21~63)</td>
<td>49.3 ± 10.5 (21~72)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td>Male: 79; Female: 29</td>
<td>Male: 28; Female: 8</td>
<td>Male: 42; Female: 19</td>
</tr>
<tr>
<td><strong>Body weight (WT) kg</strong></td>
<td>58.8 ± 5.0 (45~72)</td>
<td>59.4 ± 4.7 (50~72)</td>
<td>58.7 ± 4.5 (50~69)</td>
</tr>
<tr>
<td><strong>Serum Creatinine (Scr) µmol×L⁻¹</strong></td>
<td>102.9 ± 33.0 (51~267)</td>
<td>99.0 ± 16.8 (52~128)</td>
<td>112.9 ± 41.2 (54~218)</td>
</tr>
<tr>
<td><strong>Clearance of Creatinine (CLcr) L/h</strong></td>
<td>61.9 ± 18.0 (16~142)</td>
<td>80.5 ± 24.0 (47.7~118)</td>
<td>69.0 ± 15.6 (24.4~128)</td>
</tr>
<tr>
<td><strong>Uric Acid (UA)</strong></td>
<td>316 ± 54.8 (52~473)</td>
<td>314 ± 35.4 (230~412)</td>
<td>326 ± 33.2 (272~435)</td>
</tr>
<tr>
<td><strong>Blood urea nitrogen (BUN) mmol×L⁻¹</strong></td>
<td>5.9 ± 2.5 (2.9~25.2)</td>
<td>5.8 ± 0.9 (2.9~6.8)</td>
<td>6.0 ± 2.3 (3.1~14.1)</td>
</tr>
<tr>
<td><strong>Total bilirubin (TBIL) µmol×L⁻¹</strong></td>
<td>19.0 ± 11.2 (3.1~81.2)</td>
<td>22.8 ± 15.7 (6.7~81.2)</td>
<td>18.0 ± 11.0 (3.1~69.2)</td>
</tr>
<tr>
<td><strong>Direct bilirubin (DBIL) µmol×L⁻¹</strong></td>
<td>4.83 ± 3.59 (0.1~26.2)</td>
<td>5.2 ± 1.7 (1~6.1)</td>
<td>4.5 ± 4.6 (0.1~26.2)</td>
</tr>
<tr>
<td><strong>Alanine aminotransferase (ALT) U×L⁻¹</strong></td>
<td>37.9 ± 23.7 (7~181)</td>
<td>37.9 ± 11.5 (27~74)</td>
<td>33.4 ± 20.6 (7~107)</td>
</tr>
<tr>
<td><strong>Glutamic-oxaloacetic transaminase (AST) U/L</strong></td>
<td>35.3 ± 16.4 (10~148)</td>
<td>35.9 ± 11.2 (17~71)</td>
<td>34.0 ± 23.4 (10~148)</td>
</tr>
<tr>
<td><strong>Albumin (Alb) g/L</strong></td>
<td>38.9 ± 4.0 (25~49)</td>
<td>38.8 ± 2.8 (29~44)</td>
<td>39.0 ± 4.0 (28~48)</td>
</tr>
<tr>
<td><strong>White Blood Cell (WBC)</strong></td>
<td>6.8 ± 3.6 (1.5~12.1)</td>
<td>6.2 ± 1.1 (3.4~7.6)</td>
<td>6.5 ± 1.8 (2.3~12.1)</td>
</tr>
<tr>
<td><strong>Red Blood Cell (RBC)</strong></td>
<td>7.0 ± 3.0 (2.4~10.1)</td>
<td>8.7 ± 2.4 (4.26~10)</td>
<td>6.5 ± 3.1 (2.48~10)</td>
</tr>
<tr>
<td><strong>Hemoglobin (HB)</strong></td>
<td>126 ± 16.7 (74~170)</td>
<td>128 ± 10.8 (121~160)</td>
<td>123 ± 15.9 (77~164)</td>
</tr>
<tr>
<td><strong>Hematocrit (HCT)</strong></td>
<td>0.37 ± 0.047 (0.22~0.49)</td>
<td>0.4 ± 0.03 (0.339~0.487)</td>
<td>0.4 ± 0.05 (0.23~0.46)</td>
</tr>
<tr>
<td><strong>Blood Platelet (PLT)</strong></td>
<td>188.8 ± 56.7 (5~406)</td>
<td>186 ± 41.4 (91~286)</td>
<td>197 ± 67.3 (59~406)</td>
</tr>
</tbody>
</table>
residuals and the change in the value of the OFV were noted in the model building process. Changes in the OFV approximate the $\chi^2$ distribution with the degrees of freedom (df) equal to the number of covariates introduced. A covariate was considered statistically significant when the OFV decreased by 3.84 or greater (P<0.05, df=1) after adding into the base model (forward inclusion). The full model included all covariates that show significant decrease in OFV. Hence each covariate remaining in the model was removed in turn by fixing its value as zero. This procedure was repeated until the value of the objective function failed to increase by more than the critical value of 6.63 (P<0.01, df=1) (backward elimination). Individual pharmacokinetic parameters, arithmetic means and standard deviations were calculated using the NONMEM Bayesian estimates from POSTHOC output.

Model evaluation

The stability and performance of final model was assessed through an internal validation method that involved a non-parametric Bootstrap with resampling and replacement. In this study, 600 bootstrap samples were generated, and the PPK parameters were estimated for each of the 600 samples by using the final model. The mean and standard error of parameter estimates from the bootstrap analyses were then compared with the NONMEM Bayesian estimates from the final model.

Data analysis

Model-based estimates of individual values of AUC$_{0-24}$ were calculated according to following equation

$$AUC_{0-24} = \frac{Dose}{CL_{SS}}$$

Where CL$_{SS}$ represents CL at steady state (after 1 week therapy of SRL) and “Dose” represents the SRL dosage.

Dosage of SRL needed to achieve a desired steady-state whole blood trough concentration (5-9 ng/ml) was estimated according to following equation [34]:

$$Dose = \frac{C_{SS,min} \times Vd/F \times [1 - e^{(-k_{e} \tau)}]}{e^{(-k_{e} \tau)}}$$

Where C$_{SS,min}$ is the desired steady-state whole blood trough concentration; k$_{e}$ is the elimination rate constant which can be presented as the ratio of CL/F and Vd/F; The \( \tau \) is the dosage interval.

C$_{0}$, dose corrected C$_{0}$ (C$_{0}$/D), estimated AUC$_{0-24}$ and dosage of SRL in various CYP3A5*3 genotypes were compared by one way ANOVA method. When there is statistical difference among three groups, a SNK method was used to compare the difference between each two genotype group.

Results

Patient population

The demographic and pathophysiologic data of 108 patients were described in Table 1. Seven patients received SRL as part of the primary immunosuppressive regimen. Fifteen and ten patients received SRL in combined with a reduced dosage of CsA or TAC, respectively. The other 76 patients used SRL to replace for CsA or TAC during therapy, in case of episode of graft rejection or the occurrence of toxicity. SRL dose of the patients was 1.61 ± 0.52 mg (1-4 mg).

915 SRL C$_{0}$ blood levels were monitored from various stages post-transplant. One to twenty seven samples were collected from each patient. All samples were collected 22-24 hrs after SRL administration (Most of samples were collected on the 24 hrs after administration). The level of C$_{0}$ was 6.09 ± 3.27 ng/ml. Samples were collected from 5 days to 1683 days after transplantation. There is no significant difference of C$_{0}$ or C$_{0}$/D among different period of therapy (Table 2). 36 patients received other medications potentially interact with SRL. Including cimitidine (3 patients), omeprazole (6 patients), diltiazem (5 patients), fluvastatin (3 patients), levofloxacin (5 patients), fluconazole

| Table 2. C$_{0}$ and dose corrected C$_{0}$ of SRL at various post operation date of 112 renal transplant patients |
|----------------|----------------|----------------|-------------|
| POD             | C$_{0}$        | C$_{0}$/D       | n           |
| <1 month        | 6.01 ± 2.68   | 3.72 ± 1.47     | 83          |
| 1-3 month       | 6.50 ± 3.36   | 4.25 ± 1.85     | 139         |
| 3-6 month       | 6.08 ± 2.81   | 4.20 ± 1.88     | 133         |
| 6-12 month      | 5.59 ± 2.69   | 4.16 ± 2.25     | 193         |
| <2 year         | 5.52 ± 2.40   | 4.41 ± 2.30     | 253         |
| >2 year         | 7.46 ± 5.12   | 5.32 ± 3.36     | 132         |

C$_{0}$: trough concentration; POD: post operation date; C$_{0}$/D: ratio of trough concentration and SRL dosage.
Population pharmacokinetics of sirolimus

(5 patients), itraconazole (1 patient), nifedipine (6 patients), amlodipine (4 patients).

**CYP3A5 genotypes**

Allele frequency of CYP3A5*1 and *3 allele in 108 patients was 26.8% (18.6~35.0%) and 73.2% (64.9~81.4%), respectively. There were 11, 36 and 61 patients with *1/*1, *1/*3 and *3/*3 genotype. This result was in consistent with Hardy-Weinberg equilibrium ($\chi^2=3.09$, df=2, $P=0.213$). CYP3A5 genotype had significant impact on the $C_0$ and $C_0/D$ ($P<0.01$) (Figure 1).

Figure 1. Concentration (A) and dose corrected concentration (B) of sirolimus in various CYP3A5 genotypes in 108 Chinese renal transplant recipients. #: Outliers; #: Extreme outliers; *1/*1: homogenous for wild type of CYP3A5; *1/*3: heterozygous of CYP3A5; *3/*3: homogenous for mutant of CYP3A5.

Figure 2. Sirolimus clearance estimated through Bayesian assay versus body weight (A), albumin (B) and CYP3A5 genotypes (C).
Population pharmacokinetics of sirolimus

Compartmental pharmacokinetic analysis

Different structure models (1-, 2- compartment model, with or without lag time) were tested. The best structural model consisted of a 1-compartment model by a single first-absorption process without lag time. Since SRL was administered orally, the clearance (CL/F) and volume distribution (Vd/F) included bioavailability (F).

After the forward inclusion and backward elimination step, BW, ALB, and CYP3A5*3 genotype reduced the OFV by >6.63 (P<0.01) (Figure 2) when tested as the covariates of CL/F individually against the base model (Table 3). The PPK parameters of SRL are presented in Table 4.

<table>
<thead>
<tr>
<th>No.</th>
<th>Covariate</th>
<th>Model</th>
<th>OFV</th>
<th>∆OFV</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Basic</td>
<td>CL/F=θ₁, Vd/F=θ₂, Kₐ=θ₃</td>
<td>1928.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>WT</td>
<td>CL/F=θ₁ × (1+WT/58.6 × θ₂), Vd/F=θ₂, Kₐ=θ₃</td>
<td>1919.5 -8.5 &lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>ALB</td>
<td>CL/F=θ₁ × (1+WT/58.6 × θ₂) × e^{ALB/38.9×θ₅}, Vd/F=θ₂, Kₐ=θ₃</td>
<td>1912.6 -6.9 &lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>CYP3A5</td>
<td>CL/F=θ₁ × (1+WT/58.6 × θ₂) × e^{ALB/38.9×θ₅} × e^{CYP3A5/38.9×θ₆}, Vd/F=θ₂, Kₐ=θ₃</td>
<td>1848.5 -64.1 &lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OFV: objective function value; WT: body weight; ALB: albumin; CYP3A5: genotype of CYP3A5.

The assessment of the predictive performance of the final model is represented in scatterplots of observed concentration versus population (Figure 3A) and individual predicted SRL concentrations (Figure 3B); weighted residual versus population predicted SRL concentration (Figure 3C) and time (Figure 3D), and presented in Table 4. Residuals of most concentration data were randomly distributed within 2 standard deviations (SD), which means good agreement. The average bias of SRL was 25.6% (95% confidence interval: 17.6% to 33.6%). 497 of the 600 (82.8%) bootstrap ran successfully, and the results of bootstrap were similar with those calculated by NONMEM (Table 4).

AUC₀–2₄ of SRL was calculated through Bayesian assay. The AUC₀–2₄ was 213.2 ± 85.8 ng⋅h/ml (49.2–663.3 ng⋅h/ml). AUC₀–2₄ in CYP3A5*1/ *1, *1/*3 and *3/*3 patients was 178.4 ± 36.3, 203.5 ± 55.5 and 259.5 ± 121.6 ng⋅h/ml, respectively (P<0.05) (Table 5).

Table 3. Summary of analysis models for the pharmacokinetic parameters of sirolimus

<table>
<thead>
<tr>
<th>No.</th>
<th>Covariate</th>
<th>Model</th>
<th>OFV</th>
<th>∆OFV</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Basic</td>
<td>CL/F=θ₁, Vd/F=θ₂, Kₐ=θ₃</td>
<td>1928.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>WT</td>
<td>CL/F=θ₁ × (1+WT/58.6 × θ₂), Vd/F=θ₂, Kₐ=θ₃</td>
<td>1919.5 -8.5 &lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>ALB</td>
<td>CL/F=θ₁ × (1+WT/58.6 × θ₂) × e^{ALB/38.9×θ₅}, Vd/F=θ₂, Kₐ=θ₃</td>
<td>1912.6 -6.9 &lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>CYP3A5</td>
<td>CL/F=θ₁ × (1+WT/58.6 × θ₂) × e^{ALB/38.9×θ₅} × e^{CYP3A5/38.9×θ₆}, Vd/F=θ₂, Kₐ=θ₃</td>
<td>1848.5 -64.1 &lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Population pharmacokinetic parameters of sirolimus in Chinese renal transplant recipients

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Basic Model</th>
<th>95% CI</th>
<th>Final Model</th>
<th>95% CI</th>
<th>Bootstrap</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CL/F (θ₁)</td>
<td>10.9 (0.99)</td>
<td>8.96-12.8</td>
<td>14.4 (3.48)</td>
<td>7.58-21.22</td>
<td>14.1 (5.03)</td>
</tr>
<tr>
<td>2</td>
<td>Vd/F (θ₂)</td>
<td>357 (102)</td>
<td>157-557</td>
<td>322 (64.8)</td>
<td>195-449</td>
<td>329 (88.9)</td>
</tr>
<tr>
<td>3</td>
<td>Kₐ (θ₃)</td>
<td>2.20</td>
<td>2.20</td>
<td>2.20</td>
<td>2.20</td>
<td>2.20</td>
</tr>
<tr>
<td>4</td>
<td>WT (q₄)</td>
<td>0.19 (0.30)</td>
<td>-0.40-0.78</td>
<td>0.21 (0.26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>ALB (q₅)</td>
<td>0.26 (0.18)</td>
<td>-0.09-0.61</td>
<td>0.23 (0.17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>CYP3A5 (q₆)</td>
<td>-0.30 (0.032)</td>
<td>(-0.36)(-0.24)</td>
<td>-0.30 (0.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>wCL, %</td>
<td>30.9 (18.4)</td>
<td>-5.16-66.7</td>
<td>19.6 (9.9)</td>
<td>0.20-39.00</td>
<td>19.9 (2.4)</td>
</tr>
<tr>
<td>8</td>
<td>ωVd, %</td>
<td>23.2 (17.6)</td>
<td>-11.3-57.7</td>
<td>22.6 (15.7)</td>
<td>-8.17-53.4</td>
<td>23.0 (11.2)</td>
</tr>
<tr>
<td>9</td>
<td>σ, %</td>
<td>25.7 (4.3)</td>
<td>17.3-34.1</td>
<td>25.6 (4.1)</td>
<td>17.6-33.6</td>
<td>25.7 (8.0)</td>
</tr>
</tbody>
</table>

WT: body weight; ALB: albumin.
Population pharmacokinetics of sirolimus

To maintain the desired $C_0$ range of 5-9 ng/ml, dosage for 108 patients was $1.27 \pm 0.59$ mg to $2.29 \pm 1.07$ mg. The dosages for CYP3A5*1/*1, *1/*3 and *3/*3 patients were $2.04 \pm 0.69$ mg to $3.67 \pm 1.24$ mg, $1.61 \pm 0.30$ mg to $2.91 \pm 0.80$ mg and $0.91 \pm 0.29$ mg to $1.65 \pm 0.53$ mg, respectively (Table 5). Figure 4 showed the distribution of estimated dosage of 108 patients to obtain the $C_0$ level of 5 ng/ml. The dosage of SRL had a decreasing tendency as sequence of *1/*1, *1/*3 and *3/*3.

**Discussion**

In this study, we established a 1-compartment PPK model of SRL using conventional TDM data in Chinese renal transplant patients. Various demographic, pathophysiological and genetic factors were considered.
factors were tested as covariates of the PPK model. We found CYP3A5 genotype had significant impact on CL/F of SRL.

Different PPK models have been used to describe SRL pharmacokinetics. Most studies found 2-compartment model is suitable. In a phase I study carried out in 36 kidney transplant patients from Germany, the United Kingdom and Sweden, Ferron et al. compared biexponential and triexponential model, they found biexponential model was suitable and more complex triexponential model did not significantly improve the characterization of sirolimus kinetics [25]. Dansirikul et al. established 2-compartment SRL PPK model by using 636 samples from 25 Caucasian patients, which had significant improvement in the fitness than 1-compartment model [26]. Models with or without lag time for absorption phase have been used by various studies to describe the SRL absorption. Djebli et al. found Erlang absorption model, with 3 delay-compartments best described the concentration data [15]. In the present study, all 915 samples were conventional SRL TDM data. The available information did not allow the absorption and distributive phase to be described adequately. One-compartment model is the most suitable one in simulating SRL pharmacokinetics. This model was also applied by other studies using traditional TDM data [26, 27].\( K_a \) was fixed because the difficulty in the estimation with only \( C_0 \) data.

We tested different \( K_a \) based on references [15, 25-27], and found \( K_a = 2.2 \) had lowest OFV value [26]. The CL/F and Vd/F of the basic model of the present study were 10.9 L/hr and 357 L, respectively. The results are comparable with CL/F (7.1-28.0 L/hr) [10-15] and Vd/F (11.6-1350 L) [14, 15, 29] in previous studies.

Different demographic index was tested as the covariates of PK parameters of SRL, only BW had a significant influence on SRL clearance. The result was in consistent with previous studies [25, 28]. There is wide range of BW for patients in the present study (33-72 kg). BW changed dramatically even in the same patient during therapy. We found about 4% increasing of CL/F with every 10 kilogram increasing of

---

**Figure 4.** Predicted dosage of sirolimus for patients with various CYP3A5 genotypes. \( *1/*1 \): homogenous for wild type of CYP3A5; \( *1/*3 \): heterozygous of CYP3A5; \( *3/*3 \): homogenous for mutant of CYP3A5.
Population pharmacokinetics of sirolimus

BW. Given similar dosage, patients with higher BW may have lower SRL concentrations. BW adjusted dosage is applicable to maintain the SRL concentration in therapeutic range.

According to previous study, CL/F was found to associate with hematocrit [29] and serum lipid level [14, 27]. We found the addition of ALB on the model caused OFV decreased for 6.76 points. In the present study, for patients with 1 fold lower ALB over average level, there will be 17% increasing of CL/F, which showing a modest effect on CL/F. The result suggested that in patients with hypoalbuminemia, higher dosage of SRL may be administered. The binding rate of SRL with ALB is as high as 97%. The strong binding of SRL with ALB could decrease SRL extraction through the liver. Patients with very low level of albumin may have an elevated SRL free concentration, which caused the more rapid metabolism and elimination of SRL. On the other hand, it has been reported that the blood clearance of drugs with an intermediate hepatic extraction ratio are expected to be sensitive to change in plasma binding. Although there was no study showed the relationship between ALB and CL of SRL, the significant influence of ALB on the clearance was shown in other immunosuppressive agents with high binding rate with ALB, including mycophenolic acid [35, 36] and tacrolimus [37]. Genetic polymorphism of drug metabolism enzymes and drug transporters are considered as an important cause of PK variation [38]. CYP3A5 is the most important enzyme which metabolizes SRL. The most important SNP of CYP3A5*3 is 6896A>G mutation in CYP3A5 intron 3, which results in a splice defect of the mRNA and produces an unstable and nonfunctional protein [19, 20]. Miao et al. [39] studied the influence of genetic polymorphism on the SRL trough concentration in 50 Chinese renal transplant patients, they found trough concentration of *3/*3 was significantly higher than patients with *1 allele (P<0.05). Djebli et al. established PPK model based on 22 renal transplant patients after 1, 2 weeks and 1, 3 months therapy with SRL and CYP3A5 genotype was an important covariate of SRL CL/F. The results showed patients with *3/*3 genotype had one fold lower CL/F than patients carried *1 allele (14.1 vs 28.3 L/h). CYP3A5 genotype was not considered as a covariate in many other PPK studies on SRL. In the present study, we screened CYP3A5*3 allele as covariate in a relatively large Chinese renal transplant patient population. We found SRL CL/F in CYP3A5*3/*3 was 53.7% and 73.3% of *1/*1 and *1/*3 patients. By introducing CYP3A5 genotype as covariate of CL/F, OFV decreased for 75.3. The IIV of CL/F decreased from 30.9% to 19.6%. It seemed the CYP3A5 genotype is an important covariate of SRL CL/F in Chinese renal transplant patients.

SRL is the substrate of CYP3A and Pgp, potential drug interactions are reported in previous studies. CsA is one of important component in the immunosuppressive regimen, CsA dosage or C0 was found to associate to CL/F of SRL [14, 26, 27]. In the present study, most patients used SRL to replace for CsA or TAC in the immunosuppressive regimen. Only 15 patients obtained reduced dosage of CsA simultaneously, 85 C0 samples were obtained. We tested the CsA dosage and C0 as the covariate of SRL CL/F. However, no significant impaction of CsA on SRL was found. The reason may be that limited patients (14.7%) received CsA simultaneously, and small part of samples (9.3%) was obtained. Besides, patients in the present study received a reduced dosage to maintain the CsA C0 level in the range of 50-100 ng/ml. Thus the influence of CsA on CL/F of SRL may not be significant. For similar reason, no significant influence of TAC on SRL PK in 10 patients received reduced dosage of TAC was found. Beside immunosuppressive agents, 36 patients combined using drugs may alter the absorption and metabolism of SRL in certain period during therapy. As the time of administration, duration and dosage of the drugs introduced was variable, it is hard to test the co-administration of these drugs as a covariate. By testing the model with or without the patients who had co-administration certain drug, we found there is no significant change in the parameters between different models, thus drug interaction was not considered as a covariate in this model. The reason may be that co-administration of these drugs are occasionally given. The patients and duration of drug using are limited.

It is accepted that AUC0-24 was sensitive predictor of outcomes such as acute rejection episodes and chronic rejection. Full time-point pharmacokinetic profiles were impractical because of the increased inconvenience and costs of patients. C0 was used as TDM index for its good correlation with SRL AUC0-24 and convenience. In the present study, the PPK model
Population pharmacokinetics of sirolimus

was established based on the conventional TDM data collected retrospectively. Individualized SRL AUC_{0-24} was estimated by using Bayesian methods. The correlation between predicted AUC_{0-24} and C_0 of SRL was comparable with previous study (r^2=0.377) [15]. It seemed AUC_{0-24} has a different distribution in CYP3A5*1/*1, *1/*3 and *3/*3 patients (Table 5).

SRL dosage regimen design and adjustment is crucial to reach an individualized therapeutic target. Based on established SRL PPK model, patient’s physiopathological data and TDM data, individualized pharmacokinetic parameters (ie. CL/F) can be estimated through Bayesian method. SRL dosage can be predicted to maintain the ideal therapeutic window (5-9 ng/ml). By using PPK parameters obtained by Djebli et al., Lukas et al. [40] used Monte Carlo for the simulation of probability of renal transplant patients to achieve therapeutic index after similar SRL dosage. They found after same therapeutic protocol, CYP3A5 non-expressers (*3/*3) have higher concentration, and have higher probability to reach the therapeutic range than expressers (*1/*1 and *1/*3). In the present study, we estimated the maintaining dosage of SRL by using the individualized SRL pharmacokinetic parameters through Bayesian method. To reach the target therapeutic range, the dosage for CYP3A5*1/*1, *1/*3 and *3/*3 patients were 2.23:1.77:1. It can be deduced determination of CYP3A5 genotypes may be helpful in the design and regulation of SRL therapy regimen.

The major limitation of this study was the fixed sampling time for SRL concentration. PPK studies ideally consist of concentration-time data obtained at randomized times across a dosing interval. The data collected in the present study are all C_0. Unfortunately, because of the paucity of information in the early period after administration, it was not possible to model the absorption characteristics of SRL. On the other hand, unlike other studies, we didn’t find the influence of co-administration of CsA on SRL PPK parameters, which may be attributed to the limited patients and data in the present study. Nevertheless, the model established can be used to estimate CL/F and Vd/F well and is helpful in the SRL dosage regulation.

Conclusion

In this study we established the PPK model of SRL in patients after renal transplantation from the early stage until 3 years after transplantation by using conventional TDM data. We found besides body weight, and albumin, CYP3A5*3 genotypes is also a significant covariate for describing the CL/F of SRL. On the basis of TDM and patients pathophysiological data, the initial and maintain dosage of SRL in Chinese renal transplant patient can be simulated and individualized immunosuppressive regimen can be designed.

Acknowledgements

This work was supported by Natural Science Foundation of Shanghai (Grant Number: 12-ZR1418900). The authors have no conflicts of interest that are directly relevant to the content of this study.

Disclosure of conflict of interest

None.

Address correspondence to: Er-Zhen Chen and Bing Chen, Department of Intensive Care Unit, Pharmacy, Ruijin Hospital, Shanghai Jiao Tong University, School of Medicine, 197 Ruijin Er Road, Shanghai 200025, P. R. China. Tel: + 86 (21) 64370045; E-mail: neuroman@163.com (EZC); liuqian1976@qq.com (BC)

References

Population pharmacokinetics of sirolimus


Population pharmacokinetics of sirolimus


