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Original Article

Population pharmacokinetics of sirolimus in pediatric tuberous sclerosis complex: from Real World Study

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Abstract: Different population pharmacokinetics (PPK) of sirolimus have been established in various populations. However, the sirolimus PPK model in pediatric tuberous sclerosis complex (PTSC) remains unclear. This study aimed to establish the sirolimus PPK model in Chinese PTSC. A total of eighteen Chinese PTSC patients, from Real World Study, were characterized with nonlinear mixed-effects modeling (NONMEM). Impact of demographic features, biological characteristics, and concomitant medications was evaluated. A one-compartment model with first-order absorption and elimination was determined to be the most suitable model in PTSC. Absorption rate constant (Ka) was fixed to 0.485 h^{-1}. Typical values of CL/F and V/F in the final model were 0.484 L/h and 355 L, respectively. Inter-individual variabilities in CL/F and V/F were 15.5% and 0.4%. MCH was included as a significant covariate for CL/F. No covariates notably influenced V/F. CL/F was apparent oral clearance, V/F was apparent volume of distribution, and MCH was mean corpuscular hemoglobin. The first sirolimus PPK model in PTSC patients was developed and validated.

Keywords: Pediatric, tuberous sclerosis complex, population pharmacokinetics, sirolimus, Real World Study

Introduction

Tuberous sclerosis complex (TSC) is a genetic autosomal dominant disorder characterized by hamartomas in multiple organ systems including the brain, skin, heart, kidneys, and lungs [1]. TSC results from constitutive activation of mammalian target of rapamycin complex 1 (mTORC1) due to dysfunctional corresponding regulate proteins encoded by hamartin (*TSC1*) ortuberin (*TSC2*) [2-5]. Therefore, mTORC1 inhibitors, like sirolimus, are a promising therapeutic agent indicated for TSC [6-17]. However, sirolimus has a narrow therapeutic window and displays considerable inter-individual and intra-individual variabilities in pharmacokinetics.

With population pharmacokinetics (PPK) study, pharmacokinetic information can be acquired from sparse data pooled from a large group of subjects. Moreover, the methodology of population pharmacokinetics analysis could distinguish inter-individual variability and residual unexplained confounders. Hence, PPK has greater statistical power to verify the effects of multiple factors on the pharmacokinetics of a drug compared to traditional pharmacokinetic analysis, making it possible to formulate an optimal dose schedule [18].

Several population pharmacokinetic studies have been conducted to assess the pharmacokinetic characteristics of sirolimus, including healthy volunteers [19-24], patients with hepatic impairment [25, 26], and transplant recipients [27-36]. However, the sirolimus population pharmacokinetic model in pediatric tuberous sclerosis complex (PTSC) remains unknown. The objective of this study was to investigate the population pharmacokinetics of sirolimus in PTSC and to identify factors that explain pharmacokinetic variability, aiming to optimize individualized therapy.

Materials and methods

Patients and data collection

For this research, all data were from Real World Study. A total of 18 (9 boys and 9 girls) Chinese

PTSC patients, between January 2015 and December 2017, from Children's Hospital of Fudan University, aged 2.4-17.5 years old (mean age 8.2 ± 3.84 years), were retrospectively analyzed. Drug concentration data and relevant clinical information were obtained from therapeutic drug monitoring (TDM) records and medical records, respectively. This study was approved by the Research Ethics Committee of Children's Hospital of Fudan University.

Information extracted from medical records included gender, age, alanine transaminase (ALT), aspartate transaminase (AST), creatinine (Cr), urea (UR), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), duration of treatment with sirolimus, with or without epilepsy, and concomitant drugs (topiramate, valproic acid, clonazepam, levetiracetam, lamotrigine, oxcarbazepine, huaiqi granule). This information was thoroughly checked for accuracy.

Drug administration

All patients were given oral sirolimus. The initial daily dose of sirolimus was 1 mg. Sirolimus dosage was later adjusted according to clinical efficacy and adverse effects as well as its trough concentration in TDM. Blood samples were drawn until sirolimus concentrations reached steady state.

Analytical method

Whole blood concentrations of sirolimus were measured by Emit 2000 Sirolimus Assay (Siemens Healthcare Diagnostics Inc, Newark, USA), linear over the range of 3.5-30 ng/mL, according to manufacturer protocol.

Population pharmacokinetic modeling

Data were analyzed with a nonlinear mixed-effects model (NONMEM) computer program (version VII; ICON Development Solutions, Ellicott City, MD, USA). First-order conditional estimation method with interaction (FOCE-I) option was used to estimate PK parameters and variability. One-compartment model with first-order elimination was used to describe the absorption phase since all sirolimus concentrations in this research were trough concentrations. Bioavailability (F) and absorption with a lag time could not be estimated because sirolimus was orally administered and sirolimus concentration data were insufficient. Therefore, PK parameters were comprised of apparent oral clearance (CL/F) and apparent volume of distribution (V/F). Absorption rate constant (Ka) of the model was fixed to 0.485 h^{-1}, according to literature study [37] and research.

Random effects model

Inter-individual variability in PK parameters was explored using additive, proportional, and exponential error models. Residual error variability was evaluated with additive, proportional, exponential, and mixed error models.

Covariate model

To explain variability on PK parameters, correlation was investigated between covariates and all PK parameters for which inter-individual variability was estimated. Potential covariates included gender, age, ALT, AST, Cr, UR, HCT, HGB, MCH, MCHC, duration of treatment with sirolimus, with or without epilepsy, and concomitant medications. The covariate model was established in a stepwise manner. To compare hierarchical models, a likelihood ratio test was adopted. Changes in objective function values (OFV) caused by the inclusion of a covariate is proportional to twice the negative log likelihood of the data and approximates a Chi-square distribution. In univariate analysis, a decrease in OFV > 3.84 ($P < 0.05$, degree of freedom = 1) was used as a criterion for inclusion of the covariate in the base model. Significant covariate-parameter relationships were reserved in the model. When a full regression model was built, the model was further testified by dropping the covariate from each parameter one at a time to acquire the final model. An increase in OFV > 6.64 ($P < 0.01$, degree of freedom = 1) was used as a criterion for retaining significant covariate-parameter relationships in the model.

Model validation

An internal validation method of bootstrap was applied to evaluate the stability and reliability of final parameter estimates. Bootstrap was generated by repeated random sampling with replacement from the original data. This procedure was performed with software package Wings for NONMEM and repeated 2000 times

Table 1. Demographic data of patients included in population pharmacokinetic

Characteristic	Mean ± SD	Median (range)
Gender (boys/girls)	9/9	/
Epilepsy (yes/no)	12/6	/
Age (years)	8.20 ± 3.84	8.95 (2.4-17.5)
Duration of treatment with sirolimus (days)	114.73 ± 113.35	56.5 (23-427)
Alanine transaminase (IU/L)	14.97 ± 34.18	8 (1-195)
Aspartate transaminase (IU/L)	22.25 ± 15.53	19.5 (5-96)
Creatinine (μmol/L)	37.02 ± 11.32	34.5 (16-64)
Urea (mmol/L)	4.36 ± 1.38	4.15 (2.3-7.2)
Hematocrit (%)	37.93 ± 3.27	37.9 (29.5-46.4)
Hemoglobin (g/L)	127.68 ± 11.59	127.5 (96-162)
Mean corpuscular hemoglobin (pg)	28.13 ± 1.69	28 (23.5-31)
Mean corpuscular hemoglobin concentration (g/L)	336.77 ± 9.52	336.5 (319-355)
Concomitant drug		
Topiramate (yes/no)	3/15	/
Valproic acid (yes/no)	7/11	/
Clonazepam (yes/no)	1/17	/
Levetiracetam (yes/no)	1/17	/
Lamotrigine (yes/no)	1/17	/
Oxcarbazepine (yes/no)	5/13	/
Huaiqi granule (yes/no)	1/17	/

Table 2. Change of objective function values of covariate analysis

	Model description	OFV	ΔOFV	*P*-value
	Base model	71.119	/	/
Inclusion	Influence of MCH on CL/F	60.136	-10.983	$P < 0.05$
	Influence of LAM on V/F	55.408	-4.728	$P < 0.05$
	Full model	55.408	/	/
Elimination	Eliminate MCH on CL/F	65.578	10.17	$P < 0.01$
	Eliminate LAM on V/F	60.136	4.728	$P > 0.01$

Abbreviations: OFV, objective function values; MCH, mean corpuscular hemoglobin; LAM, Lamotrigine; CL/F, apparent oral clearance; V/F, apparent volume of distribution.

with different random draws. Medians and 2.5-97.5% percentiles of bootstrap result set parameters were compared to the final PK parameter estimates. Predictive performance of the final model was also evaluated by a prediction-corrected visual predictive check.

Results

Data collection from Real World Study

A total of 18 Chinese PTSC patients (9 boys and 9 girls) were available for population modeling. Patient characteristics and drug combinations are summarized in **Table 1**.

Modeling

A one-compartment model with first-order absorption and elimination was best fitted to the data. 0.485 h^{-1} was a reasonable value for Ka. PK parameters of sirolimus, CL/F, and V/F were estimated by NONMEM. Inter-individual variability and residual variability were best described by exponential and mixed error models, respectively. All covariates were analyzed in the present study and only the following covariate showed statistical significance on PK parameters: MCH on CL/F. No covariates notably influenced V/F. Changes of the OFV are shown in **Table 2**. Final covariate models were as follows: $CL/F = \theta_{CL/F} \times EXP(\theta_{MCH} \times MCH)$; $V/F = \theta_{V/F}$. Where $\theta_{CL/F}$ and $\theta_{V/F}$ were typical population values of CL/F and V/F, respectively; θ_{MCH} was the coefficient of the MCH; MCH was mean corpuscular hemoglobin.

Validation

Goodness-of-fit plots of the final model (**Figure 2**) compared with base model (**Figure 1**) generated by R (version 3.4.2) are shown. Parameter

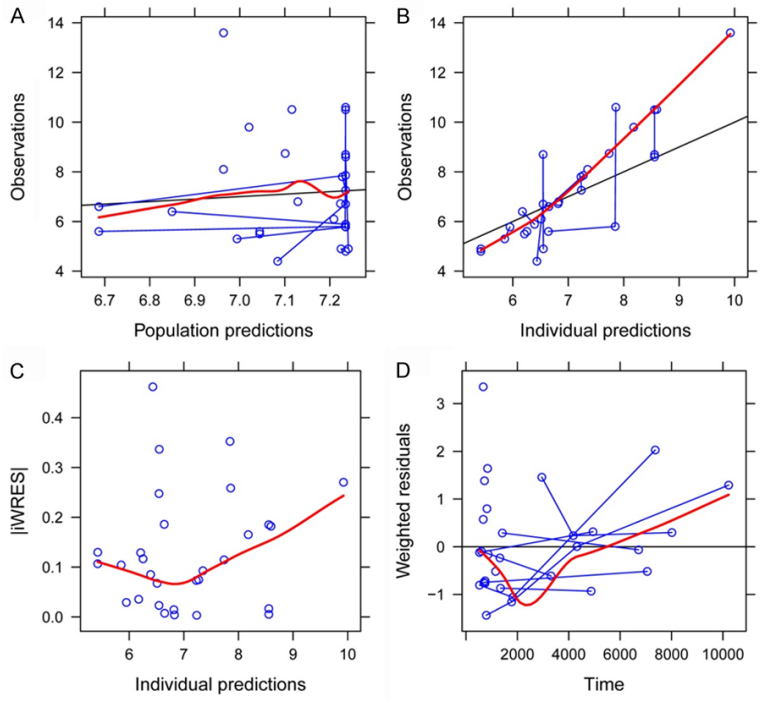

Figure 1. Goodness-of-fit plots of the base population model. (A) observations vs. population predictions (B) observations vs. individual predictions. (C) absolute value of weighted residuals vs. individual predictions (D) weighted residuals vs. time.

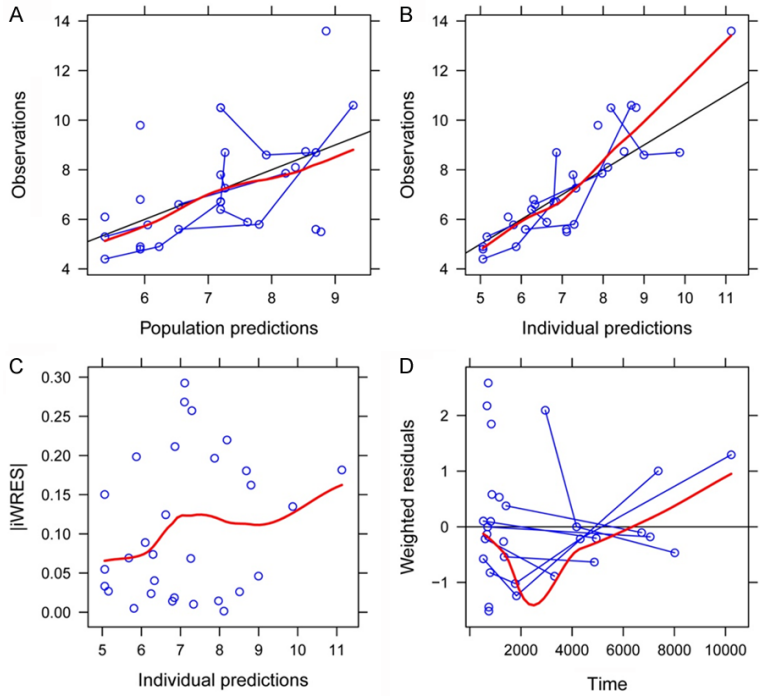

Figure 2. Goodness-of-fit plots of the final population model. (A) observations vs. population predictions (B) observations vs. individual predictions. (C) absolute value of weighted residuals vs. individual predictions (D) weighted residuals vs. time.

estimates of final model and bootstrap validation are shown in **Table 3**. Median values of the parameter estimate from bootstraps were close to respective values from the final population model, indicating that estimates for PK parameters in the final population model were accurate and the model was reliable. A prediction-corrected visual predictive check plot of the final model is shown in **Figure 3**. Most observed concentrations are within 90% prediction intervals from the simulation data, indicating that prediction-corrected concentrations were well predicted by the final model.

Discussion

The molecular connection between TSC and mTOR has led to the clinical use of allosteric mTOR inhibitors for treatment of tuberous sclerosis [38]. Therefore, sirolimus, an mTORC1 inhibitor, has become an effective therapeutic agent for TSC [6-17].

The present study is the first population pharmacokinetic analysis of sirolimus in PTSC patients. Although the TDM of sirolimus was not originally designed to investigate pharmacokinetic characteristics of sirolimus, the population approach provides a powerful tool to extract useful information from sparse sampling data [39]. Therefore, it could help optimize the use of sirolimus to achieve desirable therapeutic concentrations. Additionally, it was ethically suitable in studying pediatric patients prohibited excessive blood sampling compared with traditional pharmacokinetic stud-

Table 3. Parameter estimates of final model and bootstrap validation

Parameter	Estimate	Bootstrap		Bias
		Median	95% Confidence interval	
CL/F (L/h)	0.484	0.543	[0.121, 7.194]	0.121
V/F (L)	355	392.5	[1.837, 1890]	0.106
Ka (h^{-1})	0.485 (fixed)	--	--	--
θ_{MCH}	0.0837	0.068	[-0.01, 0.133]	-0.191
$\omega_{CL/F}$	0.155	0.114	[0.003, 0.264]	-0.265
$\omega_{V/F}$	0. 004	0	[0, 0.334]	-1
σ_1	0.173	0.157	[0.004, 0.223]	-0.092
σ_2	0.007	0	[0, 1.172]	-1

95% confidential interval was displayed as the 2.5th and 97.5th percentile of bootstrap estimates. CL/F, apparent oral clearance (L/h); V/F, apparent volume of distribution (L); Ka, absorption rate constant (h^{-1}); θ_{MCH} was the coefficient of the MCH; $\omega_{CL/F}$, inter-individual variability of CL/F; $\omega_{V/F}$, inter-individual variability of V/F; σ_1, residual variability, proportional error; σ_2, residual variability, additive error; Bias, prediction error, Bias = (Median-Estimate)/Estimate.

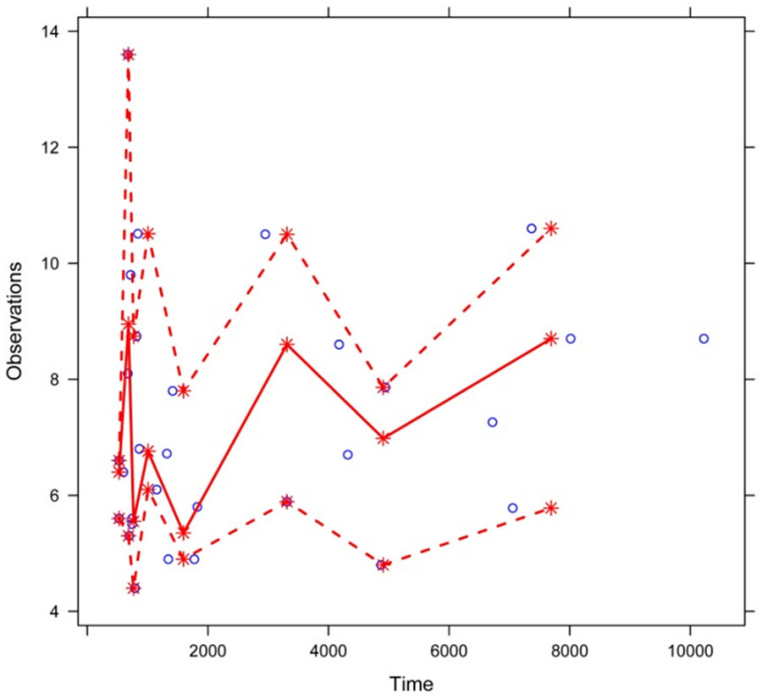

Figure 3. Prediction-corrected visual predictive check (VPC) for the final model. Solid line represents the median of observations and the dashed lines represent upper and lower limits of 90% confidence interval of observations.

ies [40]. Also, this sirolimus PPK model has clinical value in predicting the pharmacokinetic process in individual pediatric patients with TSC.

Several previous studies have shown that sirolimus fits a two-compartment better than one-compartment model [33, 35, 36]. TDM data in this study only supported a one-compartment model. Model misspecification may lead to biased estimates of pharmacokinetic parameters. However, a recent study [41] by Kowalski and Hutmacher found that biases in the estimates of CL/F and apparent steady-state volume of distribution (Vss/F) were all near zero, by Monte Carlo simulation in such situations, which fits a one-compartment oral model to data that were simulated using a two-compartment model.

In this present study, a one-compartment model with first-order elimination was used to describe the absorption phase, as all sirolimus concentrations were trough concentrations. Ka found was best fixed to 0.485 h^{-1}, slower than that in Chinese adult renal transplant patients [37] whose Ka was fixed to 0.752 h^{-1}. Typical values of CL/F and V/F in the final model were 0.484 L/h and 355 L, respectively. Inter-individual variabilities in CL/F and V/F were 15.5% and 0.4%. This model also tested various covariates on different parameters, with the following covariate determined to be statistically meaningful: MCH on CL/F. This may indicate that MCH influenced the pharmacokinetic process of sirolimus in PTSC patients. No covariates significantly influenced V/F. Additionally, of the 18 subjects in the study, twelve patients were concurrently diagnosed with seizure and treated with following agents: topiramate, valproic acid, clonazepam, levetiracetam, lamotrigine, and oxcarbazepine. However, no significant drug-drug interactions were found in analyzing sirolimus pharmacokinetics.

Sirolimus is a substrate of both CYP3A enzymes and P-glycoprotein. Polymorphisms in *CYP3A* and P-glycoprotein might be associated

with inter-individual variations in sirolimus dose determination [34, 42]. However, its pharmacogenomic consideration has not been verified in clinical use. This study was from Real World Study, retrospectively, which means genotyping was not routinely performed in our population. Whether inclusion of genotyping in this model would better explain the variabilities of sirolimus in PTSC should be further studied.

In conclusion, this population pharmacokinetic model of sirolimus in PTSC patients was established using retrospective and routinely monitored data from Real World Study.

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Disclosure of conflict of interest

None.

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