

Population pharmacokinetic analysis of the microemulsion formulation of cyclosporin A (Neoral®) in renal transplant patients

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Aims: Optimizing the use of Cyclosporin A (CsA) has been a challenge due to its large inter- and intraindividual variability in pharmacokinetic (PK) disposition and its narrow therapeutic window. The aim of the current study was to develop a population PK model to characterize CsA steady-state PKs in renal transplant patients and to apply it to the Bayesian estimation of drug exposure using limited sampling. **Method:** The steady-state PKs of CsA were collected from 60 renal-transplant patients grafted for at least 3 months. Population PK analysis was performed using the nonlinear mixed-effect approach, as implemented in NONMEM (version V). For the final PK model analysis, the bootstrap method with replacement was applied to construct confidence intervals (CIs) for the parameters. Using the *post hoc* Bayesian option individual PK parameters were estimated in the validation set using the final PK model. **Results:** The population mean for clearance (CL/F), volume of distribution (V/F), and first-order absorption rate constant (Ka) were 19.9 l/h (95% CI: 13.7–28.1 l/h), 191 l/h (95% CI: 149–378 l/h), and 1.56 l/h (95% CI: 1.11–1.67 l/h), respectively. Interindividual variability of CL/F, V/F, and Ka were 85.4, 55.6 and 90.5%, respectively. Residual variability was 44.9%. **Conclusion:** The developed population PK model adequately described the CsA profile in renal-transplant patients. Bayesian estimation using this model provided reasonably good estimates on individual patient CsA exposure.

Cyclosporin A (CsA) is one of the most widely used immunosuppressive agents in organ transplantation. Optimizing the use of CsA has been a challenge due to its large inter- and intraindividual variability in absorption kinetics and bioavailability, in addition to its narrow therapeutic window [1–9]. Studies have shown that CsA exposure is the most sensitive predictor of outcomes such as acute rejection episodes and graft loss at 1 year post-transplantation in adult renal transplant recipients [10–12]. Nephrotoxicity occurs more frequently with CsA concentrations above the desired drug exposure [4]. The microemulsion formulation of CsA (Neoral®) has been demonstrated to have a less variable pharmacokinetic (PK) disposition as a result of better absorption and less influence on the rate and extent of absorption by food, liver and pancreas function [13–17].

Various PK strategies have been proposed for CsA monitoring including trough-level monitoring (C_0), single-point concentration at 2 h post dose (C_2), and various sampling algorithms using two to five sampling time points. The results, however, are variable and center-specific [18,19]. The area under the concentration–time curve (AUC) has been demonstrated

as a more precise predictor of acute rejection and graft survival compared with other PK parameters, including trough levels [12,19]. Recent studies suggest that AUC monitoring can be simplified using a limited sampling strategy in a routine clinical setting. Several multiple linear regression-based methods have been reported to estimate CsA AUC using limited sampling [18,20–22]. The performance of the multiple linear regression-based methods are highly dependent on fixed and accurate sampling time; the variability associated with sampling time, both when patients take their medication before a clinic visit as well as sampling time post-dosing during a clinic visit, can significantly impact the accuracy of drug exposure prediction. Recently, several authors have shown that population PK-based analysis; especially Bayesian estimation on the basis of population PK models, can provide a good estimation of individual patient CsA exposure from limited sampling, which is feasible for the routine CsA therapeutic drug monitoring (TDM) setting [17,23–26].

The aim of the present study was to develop a CsA population PK model that enables the prediction of individual steady-state PK exposure on the basis of Bayesian estimation.

Keywords: Bayesian estimation, cyclosporin A, Neoral®, organ rejection, population pharmacokinetics, renal transplant patients



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Table 1. Patient characteristics.

Characteristics	Mean ± SD	Range
Age (years)	55 ± 12	16–96
Body weight (pounds)	170 ± 35	30–168
Dose (mg/12 h)	134 ± 68	50–425

SD: Standard deviation.

Patients, materials & methods**Patient population & data collection**

The steady-state PKs of CsA was collected from 60 renal transplant patients grafted for at least 3 months. The enrolled patients were provided with written informed consent and their characteristics are summarized in Table 1. All subjects received stable doses of CsA twice daily for at least 4 weeks. Samples were collected before (trough), and at 0–3, 4–7 and 7–12 h after dosing. Accurate documentation of dosing and sampling times were recorded. Whole blood samples were analyzed for CsA concentration using the monoclonal fluorescence polarization immunoassay (FPIA) on a TDx analyzer (Abbott Laboratories) according to the manufacturers recommendations. The interday coefficients of variation (CV) for the quality-control samples of 71, 252 and 739 ng/ml were 9.8, 5.5 and 4.0% respectively. The data set was divided into two groups: an index set of 50 patients and a validation set of ten patients.

Population pharmacokinetic analysis in the index set

The population PK analysis was performed using the nonlinear mixed-effect approach as implemented in NONMEM V [27]. One- and two-compartment open models with first-order

(FO) absorption and elimination were compared with the fit of the data. The FO estimation method was used for initial modeling, and the FO conditional estimation with interaction (FOCEI) method was attempted on the final model to improve fitting. Various covariance structures with different variability were modeled and tested by applying the OMEGA BLOCK option in NONMEM.

Once the final structural and variance model was selected, the covariate effects on parameters were initially examined graphically using generalized additive model analysis. Once significant covariates were identified, a stepwise forward selection and backward elimination approach was applied for final covariate model development.

Model selection was based on various goodness of fit indicators, including comparisons based on the minimum objective function value (MOFV), visual inspection of diagnostic scatter plots and evaluation of estimates of population fixed and random effect parameters.

Bootstrapping & confidence interval

For the final PK model analysis, the bootstrap method with replacement was applied to construct confidence intervals (CIs) for the parameters. Separate estimations were performed for 300 bootstrapped data sets. The 2.5 and 97.5 percentile of the set of estimates defined the lower and upper limit of the 95% CIs, respectively, for each parameter and its corresponding variability.

Bayesian analysis in the validation set

Bayesian estimation was performed by conducting the final model NONMEM run with the post hoc option on the validation data set using the final estimates obtained from the index data set as starting values and employing the MAX EVAL = 0 option on the \$EST statement. The predictive performance of Bayesian estimates was evaluated by comparing predicted and observed concentrations in all subjects. Bias was estimated by mean predictive error (me) and precision of the predictions was estimated by root mean squared prediction error (rmse) according to the following equations:

$$me = \frac{1}{N} \sum_{i=1}^N [(C_{PRED} - C_{OBS}) / C_{OBS}] \times 100$$

$$rmse = \frac{1}{N} \sum_{i=1}^N \sqrt{\{[(C_{PRED} - C_{OBS}) / C_{OBS}] \times 100\}^2}$$

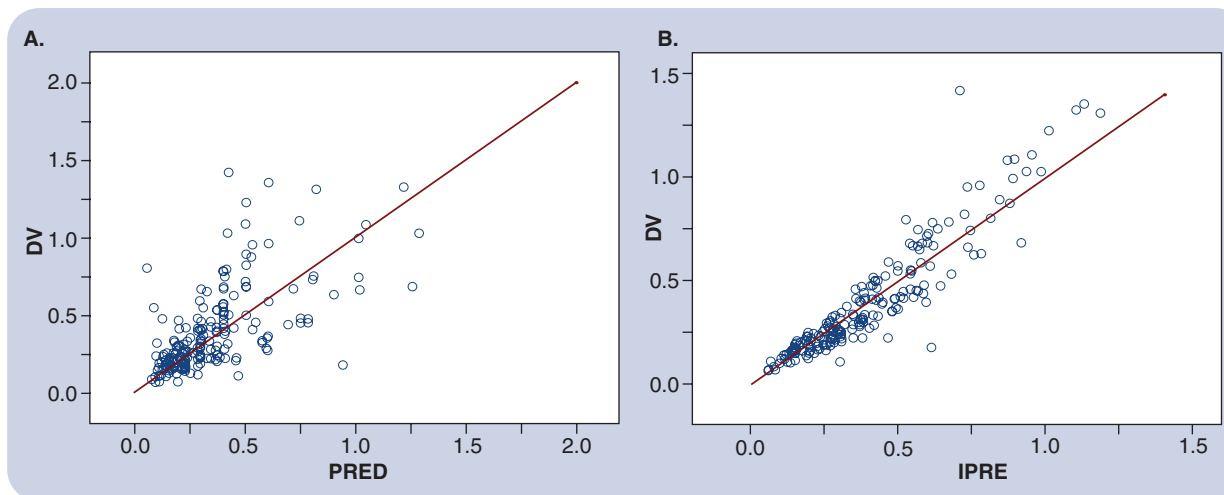
Table 2. Parameter estimates for population pharmacokinetic model of CsA.

Parameter	Parameter estimate (%CV)	95% CI SE derived	95% CI* Bootstrap derived
CL/F (l/h)	19.9 (9.4%)	16.2–23.6	17.3–28.1
V/F (l)	191 (18.9%)	120.2–261.8	149–378
Ka (l/h)	1.56 (4.8%)	1.41–1.71	1.11–1.67
IIV in CL/F (%)	85.4 (46.2%)	-	-
IIV in V/F (%)	55.6 (76.7%)	-	-
IIV in Ka (%)	905 (60.7%)	-	-
Residual error	44.9 (15.9%)	-	-

*Confidence interval (CI) was calculated from 300 bootstrapped replicates.

CL/F: Clearance rate; Ka: Absorption rate constant; SE: Standard error;

V/F: Volume of distribution.

Figure 1. Model-predicted concentration scatter plots.

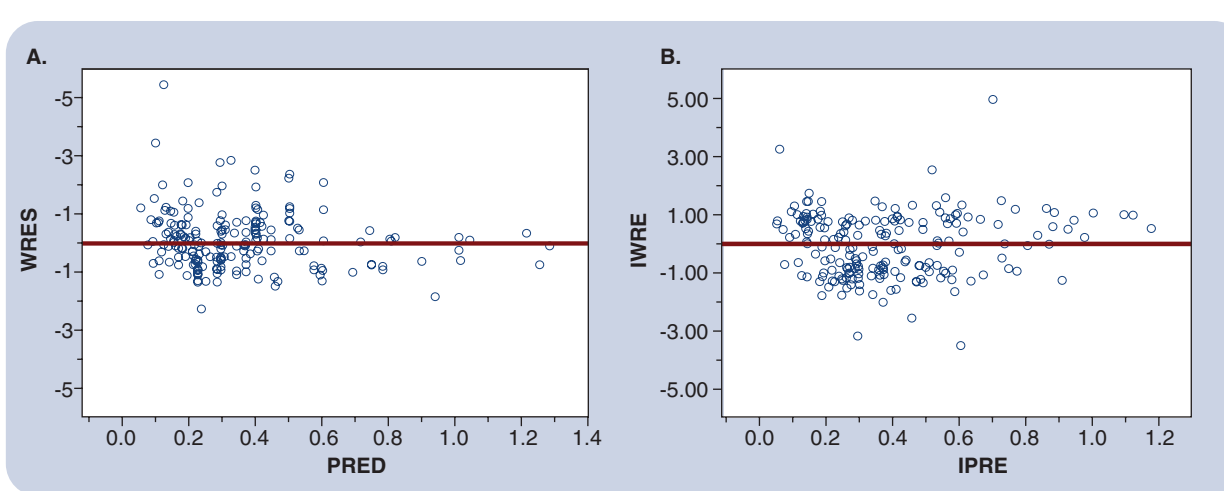
Scatter plots of **(A)** population model-predicted concentration (PRED) versus observed concentrations (DV) and **(B)** individual model-predicted concentrations (IPRE) versus DV.

Results & discussion

A one-compartment model with FO absorption and elimination, as well as interindividual variability on clearance (CL/F), volume of distribution (V/F), and the FO absorption rate constant (Ka), was chosen as the base structural model based on the likelihood ratio test. The addition of full OMEGA BLOCK structure significantly decreased the objective function value. An exponential error model and a proportional error model were found to best describe the interindividual variability in PK parameters and the

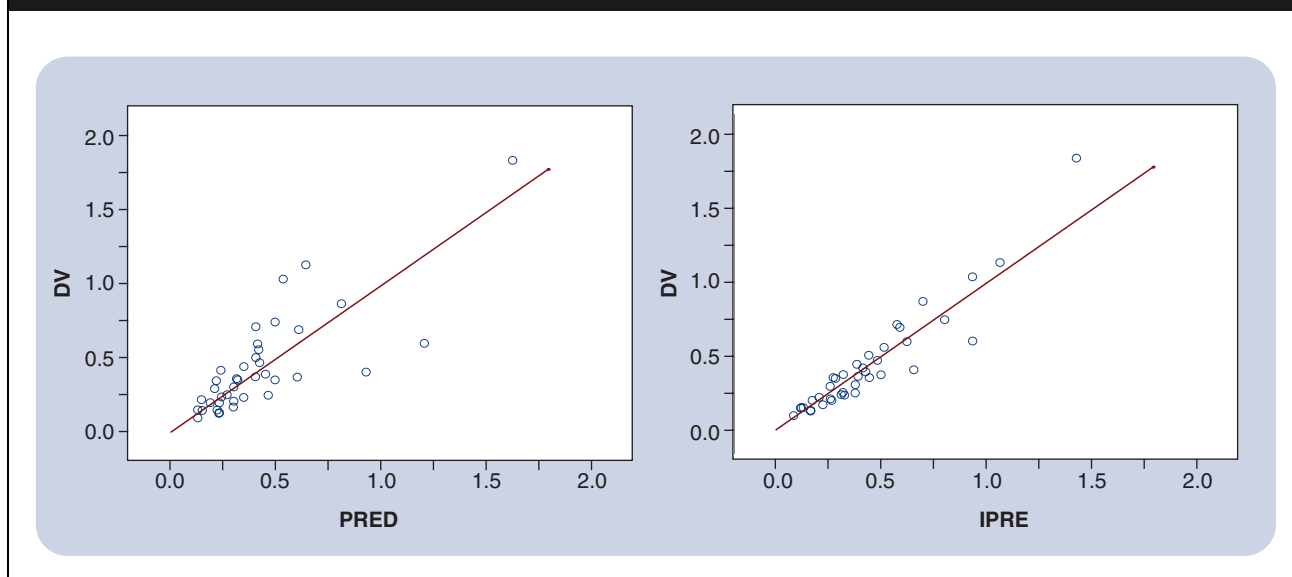
residual variability, respectively. No covariate demonstrated significant influence on CL/F, V/F, and Ka. FOCEI did not improve fitting, therefore, FO estimation was used in the final model.

For the final model, the bootstrap method with replacement was applied to construct confidence intervals CIs for the parameters. Separate estimations were performed for 300 bootstrapped data sets to calculate the 2.5 and 97.5 percentile of the set of estimates that define the low and upper limit of the 95% CIs, respectively, for each parameter. The final PK

Figure 2. Model-predicted concentration scatter plots.

Scatter plots of **(A)** population model-predicted concentration (PRED) versus weight residue (WRES) and **(B)** individual model-predicted concentrations (IPRE) versus individual weight residue (IWRES).

Figure 3. Scatter plots of the population predicted (PRED) and Bayesian estimated concentrations (IPRE) versus observed concentrations (DV) in the validation set.



parameter estimates are summarized in Table 2. The CL/F, V/F, and K_a rates were 19.9 l/h (95% CI, 13.7–28.1 l/h), 191 l/h (95% CI, 149–378 l/h), and 1.56 l/h (95% CI, 1.11–1.67 l/h), respectively. These values are comparable to the values reported for kidney transplant patients. In agreement with previous studies, we also observed a high interindividual variability, as evidenced by interindividual variability in CL/F and V/F of 85.4 and 55.6%, respectively. A large intersubject variability in oral absorption rate constant (K_a) estimated by the population PK model was observed. The magnitude of the variability expressed as coefficient of variation (%CV) is 905%, which is likely related, at least in part, to the small number of data points during the early absorption phase, beside the reflection of variable and erratic absorption characteristics associated with CsA. The residual variability was 44.9%.

A number of PK models including a two-compartment model with FO absorption with a lag time, or a model with γ distribution absorption kinetics have been used to describe CsA PKs. However, the selection of a model is largely dependent on the PK sampling scheme. For example, extensive sampling during the early absorption/distribution phase is required in order to justify a more complex model. In our case, a one-compartment model was selected to characterize the CsA PKs for routine TDM. The population PK analysis was

reasonably robust as evident by the plots of the observed versus the population and individual predicted CsA concentration (Figure 1). No major bias was observed with the residual plots as illustrated in Figure 2.

Population parameters obtained with the index set were employed as priors for Bayesian estimation in the validation set to estimate PK disposition in each patient. As shown in Figure 3, concentrations predicted with Bayesian estimation showed a reasonably good correlation with observed concentrations. Comparing the Bayesian estimated with the observed concentrations, the me value was 5.9% and the rmse was 19.9%.

CsA is one of the most widely used immunosuppressive agents in organ transplantation. Due to large inter- and intraindividual variations, its PK behavior in patients is still difficult to predict. Dose optimization is still mainly performed on a trial and error basis. In this paper, we developed a population PK model, which was designed to help physicians in adjusting patient-specific CsA dosing regimens.

The population PK model described resulted in an adequate fit of the observed data, and the estimated population PK parameter values are in agreement with those published previously. Moreover, the Bayesian method using sparse data on the basis of the final population PK model gave a good prediction of individual CsA exposure, thus further demonstrated that population PK-based Bayesian analysis can be a useful tool for routine TDM of CsA.

Conclusion

In summary, the developed population PK model adequately described the CsA profile in renal transplant patients. Further evaluation by bootstrapping showed that the population PK

model was stable. Bayesian estimation using this model provided reasonably good estimates on CsA exposure. This provides further evidence that population PK-based Bayesian analysis can be a useful tool for routine TDM of CsA.

Highlights

- Optimizing the use of cyclosporine has been a challenge due to its large variability in pharmacokinetics (PKs) and narrow therapeutic window.
- A population PK model has been developed to characterize the pharmacokinetics of cyclosporine after multiple oral administration of the microemulsion formulation, Neoral®, in kidney transplant patients.
- Bayesian estimation using this developed population PK model provided reasonably good estimates of cyclosporine exposure in individual patients.
- The results of this study suggests that population PKs based Bayesian analysis can be a useful tool for routine therapeutic drug monitoring of cyclosporine.

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