ABSTRACT:

Population pharmacokinetics of cyclosporine (CsA) in clinical renal transplant patients has been reported in the present study. A total of 2548 retrospective drug monitoring data points were collected from 120 renal transplant patients receiving CsA. Population modeling was performed using the NONMEM (nonlinear mixed-effect modeling) program, using a one-compartment model with first-order absorption and elimination. The final regression model for CsA clearance (CL/F) with the influence of six significant covariates, comprising postoperative days (POD), total bilirubin level (TBIL, micromolar concentration), current body weight (CBW, kilograms), age (years), concurrent metabolic inhibitors of cyclosporine (INH), and hematocrit (HCT, percentage), has been established and expressed as CL/F = 28.5 – 1.24 · POD – 0.252 · (TBIL – 11) + 0.188 · (CBW – 58) – 0.191 · (Age – 42) – 2.45 · INH – 0.212 · (HCT – 28) (liters per hour). The values in parentheses represent the median level for each of the corresponding covariates. The population estimates for CL/F (28.5 l/h), V/F (volume of distribution, 133 l), and interpatient variability (CV% = 19.7%) for CL/F were achieved, respectively. The population model was further validated by internal and external approaches, and was demonstrated to be effective and stable. Moreover, simulation was conducted to facilitate the individualized treatment based on patient information and the final model.

Cyclosporine (CsA) has been introduced into organ transplantation since the early 1980s, and has been shown to largely reduce the rate and severity of graft-versus-host disease and to increase success in graft and survival of patients (Hesselink et al., 2004). Today, CsA has become the backbone of immunosuppression in clinical organ transplantation (Kyriakides and Miller, 2004). As a result, short-term and medium-term kidney allograft survivals have greatly increased. However, CsA application has exhibited a high degree of interindividual and intraindividual variability in pharmacokinetic and/or pharmacodynamic aspects. Furthermore, the therapeutic window (range of drug concentration for desired therapeutic effect) with acceptable tolerability is very limited (Armstrong and Oellerich, 2001; Abraham, 2004). Levels below the window are associated with a high risk of organ rejection, whereas levels above the window correlate with side effects, such as nephrotoxicity, infection, hepatotoxicity, and tumor (Kasiske et al., 1988).

Many clinical pharmacokinetic studies of CsA have been conducted using ordinary pharmacokinetic methods, which were focused on individual parameter estimates, with multiple blood-sampling points (Banner et al., 2002; Trompeter et al., 2003). However, the pharmacokinetic properties of CsA changed greatly between patients and between investigations. It has been difficult to predict its disposition in a specific individual, although pharmacokinetics and pharmacodynamics of CsA have been well reported in the literature. In contrast to the traditional pharmacokinetic approach, population pharmacokinetics has great advantages in estimation of the population parameters and analysis of factors (i.e., influence of demographic parameters and physiological conditions on the pharmacokinetic parameters). The population method is robust to predict a drug’s behavior based on specific individual information and, moreover, is ideal in analyzing the sparse data commonly obtained in the clinic (Sheiner et al., 1977).

In the present study, the medical histories of 120 patients receiving renal transplant were retrospectively analyzed, and a population pharmacokinetics study of CsA in the patients was performed using NONMEM (nonlinear mixed-effect modeling). Consequently, the pharmacokinetic model was defined, using routing drug monitoring data, and could be used to improve the clinical application of CsA.

Materials and Methods

Patients and Data Collection. Plasma concentration data of CsA from 120 patients receiving renal transplantation in the past 4 years in Peking University First Hospital, Beijing, China, were collected. The patients were divided into two groups: 99 in the index group for the construction of the model and 21 in the validation group for external validation. All the patients were treated with...
coadministration of CsA, mycophenolate mofetil (or azathioprine), and corticosteroid. CsA was administered orally in the soft capsule formulation twice daily. There were three brands of CsA: Neoral (Novartis, Basel, Switzerland), Tianke (North China Pharmaceutical Group Corporation, Shijiazhuang, China) and Neocyspin (Zhongmei Huadong Pharmaceutical Co. Ltd, Hangzhou, China). Neoral and Neocyspin were composed of a microemulsion formulation in soft capsule, whereas Tianke was packed in a common soft capsule and turns into microemulsion in the gastrointestinal tract spontaneously. Among the patients, 93 of the 120 patients in the study were administered Neoral, 25 received Tianke, and 7 received Neocyspin, respectively. In addition, five patients were administered the CsA products from two different manufacturers. In a routine protocol, methylprednisolone was first given for 3 days after the operation, followed by prednisone on a once a day regimen. Methylprednisolone or dexamethasone was occasionally given for graft-versus-host disease as needed. The hepatic function and normal blood and biochemical parameters for the patients were examined and reported as normal (Table 1).

The influence of different covariates was analyzed as follows: age, height, current body weight (CBW), body mass index (BMI), sex, postoperative day, hematocrit (HCT), concurrent metabolic inhibitors of CsA (INHI), corticosteroid, liver function tests [total bilirubin level (TBIL), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase], and different CsA dosage forms (DRUG). These covariates were selected according to the previous reports and clinical common sense.

Blood Sampling and CsA Analysis. Whole blood samples were collected immediately at predose (C₀), 2 h (C₁) and every 2 or 3 days postdose according to clinical need until termination of the treatment or change to other immunosuppressants (usually tacrolimus). All samples were transferred into EDTA-Vacutainer tubes, and CsA concentrations in whole blood were measured immediately utilizing fluorescence polarization immunosassay technology (TDx; Abbott Laboratories, Abbott Park, IL), which is capable of detecting CsA concentrations with 95% confidence for the samples containing ≥25.00 ng/ml, and the values of CV were less than 4%.

Population Model Construction. Population pharmacokinetic analysis was performed using the NONMEM program (Version V, Level 1.1). A onecompartment open model with first-order absorption and elimination was used to analyze CsA data. The model consisted of an absorption rate constant (Kₐ), clearance (CL/F), and apparent volume of distribution (V/F). Since almost all retrospective data were located at the two ends of absorption phase in the study, Kₐ, the constant of absorption, was presumed to be 1.28 h⁻¹ based on literature values (Rui et al., 1995; Parke and Charles, 2000).

The model was established using the forward inclusion-backward elimination method (Mandema et al., 1992). In the first step, the population pharmacokinetic analysis was conducted without any covariates in the basic model. Scatter plots of CL/F and V/F against each covariate helped to identify the trends and the regression pattern. In the second step, each candidate covariate was screened in turn by incorporating it into the basic model to develop the intermediate and full models and by observing the decrease of the objective function value. The difference in objective function value (OFV) was kept as a χ² distribution, and an OFV value greater than 3.84, associated with a p value of 0.05, was used for statistical significance. There were also many indicators for the improvement of fit due to the addition of a parameter to the model: decrease in standard error of the parameter estimates, reduction in interpatient and intrapatient variability, agreement between the observed and predicted concentrations, reduction in weighted residuals, and uniformity of the scatter plot of weighted residuals versus predicted concentrations (Beal and Sheiner, 1989). The influences (Age, height, CBW, BMI, ALT, ALP, TBIL, GGT, and HCT) were included in the model as continuous covariates in a linear way. Discrete covariates, such as sex, INHI, DRUG, and POD, were evaluated by stepwise inclusion of scaling factors: sex = 0 for males and sex = 1 for females; INHI = 1 for those coadministered with metabolic inhibitors of CsA (diltiazem or verapamil) and otherwise, INHI = 0; DRUG = 1 for Neoral, DRUG = 1 for Neocyspin, and DRUG = 2 for Tianke; POD values are assigned in Table 2. In the final step, the influence of each covariate remaining in the full model was removed in turn, by fixing its value to zero. This process was repeated until the increase of objective function was less than the critical value of 10.83 (p < 0.001).

The statistical model used to describe interindividual variability in the pharmacokinetic parameter of CsA was expressed in eq. 1 (exponential):

$$ P_{ij} = P_{ij0} \cdot \text{Exp}(\eta_{ij}) $$

(1)

where $P_{ij}$ is the $j$th basic pharmacokinetic parameter for the $i$th individual, $P_{ij0}$ is the typical value of the $j$th population parameter, and $\eta_{ij}$ is a random variable for the $i$th individual in the $j$th parameter distributed with a mean of 0, and variance of $\omega_{ij}$.

A combined proportional and additive model was used to describe the residual variability (eq. 2):

$$ C_{OBS} = C_{PRD} \cdot (1 + e_1) + e_2 $$

(2)

where $C_{OBS}$ and $C_{PRD}$ are the observed and predicted blood CsA concentrations, respectively, $e_1$ and $e_2$ are randomly distributed terms in which each has zero mean and variances of $\sigma_1$ and $\sigma_2$, respectively.
Model Validation. The contributions of the individual on the modeling results and robustness of the final population model were assessed using internal and external validation strategies. A data-splitting method was used as internal validation (Ishibashi et al., 2003). Patients in the index group were randomly divided into 10 subpopulations and each of them consisted of 90% of the patients in the original population. Each subset was analyzed by NONMEM with the final model to obtain the parameter estimates, which were compared with those resulting from the full data set. Next, the objective function value was calculated by applying each of the 10 subset estimations into the full data set. These objective functions were compared with that from the full data set using the final model. Then a Jackknife estimate was used to calculate the 95% confidence interval (CI) for parameter estimates, which was compared with those obtained by NONMEM.

An external method was also applied to validate the final model (Sheiner and Beal, 1981). Another 21 patients were included in the validation group (Table 1). The observed concentrations were compared with the corresponding predictions by NONMEM based both on the basic and final model. Predictive performance was assessed in terms of bias [mean predicted error (ME)] and precision [mean squared prediction error (MSE) and root mean squared prediction error (RMSE)]. Moreover, standardized prediction error (SPE) was also introduced in the model validation (Serrano et al., 1999). The indicators were defined as follows.

$$\text{ME} = \frac{1}{N} \sum_{i=1}^{N} (C_{\text{PRED}} - C_{\text{OBS}})$$

$$\text{SPE} = \frac{(C_{\text{PRED}} - C_{\text{OBS}})^2}{C_{\text{OBS}}^2}$$

where $C_{\text{PRED}}$ is the predicted concentration, $C_{\text{OBS}}$ is the observed concentration, and $N$ is the number of observations.

**TABLE 4**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimates from Final Model (NONMEM Estimate)</th>
<th>Results of Internal Validation (Jackknife Estimate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL/F&lt;sub&gt;TV&lt;/sub&gt; (l/h)</td>
<td>28.5</td>
<td>28.5</td>
</tr>
<tr>
<td>V/F&lt;sub&gt;TV&lt;/sub&gt; (l)</td>
<td>133</td>
<td>134</td>
</tr>
<tr>
<td>K&lt;sub&gt;a&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt; (1/h)</td>
<td>1.28</td>
<td>1.29</td>
</tr>
<tr>
<td>f&lt;sub&gt;PEO&lt;/sub&gt;</td>
<td>1.24</td>
<td>1.29</td>
</tr>
<tr>
<td>f&lt;sub&gt;MT&lt;/sub&gt;</td>
<td>0.252</td>
<td>0.306</td>
</tr>
<tr>
<td>f&lt;sub&gt;CBW&lt;/sub&gt;</td>
<td>0.188</td>
<td>0.217</td>
</tr>
<tr>
<td>f&lt;sub&gt;SE&lt;/sub&gt;</td>
<td>0.191</td>
<td>0.158</td>
</tr>
<tr>
<td>f&lt;sub&gt;NSH&lt;/sub&gt;</td>
<td>2.45</td>
<td>2.80</td>
</tr>
<tr>
<td>f&lt;sub&gt;AC&lt;/sub&gt;</td>
<td>0.212</td>
<td>0.220</td>
</tr>
<tr>
<td>Interindividual variability (% CV)</td>
<td>(\text{CL}/F)</td>
<td>19.7</td>
</tr>
<tr>
<td></td>
<td>(K_a)</td>
<td>179</td>
</tr>
<tr>
<td>Residual error (CV% if proportional, S.D. if additive)</td>
<td>(\sigma_1) (Proportional)</td>
<td>30.8</td>
</tr>
<tr>
<td></td>
<td>(\sigma_2) (Additive)</td>
<td>42.4 (ng/ml)</td>
</tr>
</tbody>
</table>

* K<sub>a</sub> was fixed at 1.28 and the interindividual variability of V/F was fixed at 0.
where $C_{\text{OBS}}$ and $C_{\text{PRED}}$ are the observed and predicted blood CsA concentrations, respectively. S.D.($C_{\text{pred}}$) is the standard deviation in the predicted values, while $N$ is the total number of observations in the validation group.

**Trial Simulation.** Simulations were carried out by NONMEM to facilitate the individualized treatment based on patient information and the final model. CsA (175 mg) was orally administered twice a day. The value of all the key covariates was assigned according to those in the index group. A standard patient with the average values for continuous key covariate (TBIL, CBW, Age, and HCT) was simulated and compared with other patients with a different value of covariate (mean ± 2 S.D.). The probability of the covariate value falling in the interval of mean ± 2 S.D. is 95.5%, and this range is wide enough to assess the influence of each covariate. The essential information for simulation is shown in Table 3. The simulation for each situation was conducted 500 times.

**Results**

The plasma CsA concentration versus time curves were well described using a one-compartment open model. The distribution of random residual errors was expressed using a combined model (additive and proportional) to best interpret intrapatient variability. The resulting population model with the covariates of CL/F (Age, CBW, POD, TBIL, HCT, and INHI) is shown in eq. 7,

\[
\text{CL/F} = 28.5 - 1.24 \cdot \text{POD} - 0.252 \cdot (\text{TBIL} - 11) - 0.188 \cdot (\text{CBW} - 58) - 0.191 \cdot (\text{AGE} - 42) - 2.45 \cdot \text{INHI} - 0.212 \cdot (\text{HCT} - 28)
\]

in which the influence scopes (TBIL, CBW, Age, HCT) were adjusted by their respective median values determined from the database (11 μM for TBIL, 58 kg for CBW, 42 years for Age, and 28% for HCT, respectively). INHI was defined as 1 in the patients coadministered...
with metabolic inhibitors of CsA (diltiazem or verapamil), or as zero otherwise. POD value was estimated based on the postoperative day (Table 2). In addition, no statistical significance was observed in the presence of brand of CsA as a covariate on $K_a$. Interindividual variability of $V/F$ was considered as zero due to its insignificant values.

The population pharmacokinetic parameters with the model are listed in Table 4. The relative standard errors (% RSEs) of estimation for the parameters were acceptable, with a range from 3.27% to 27.2%. The interindividual variability for $CL/F$ and $K_a$ were 31.3% and 16.1%, respectively. Figure 1A shows the relationship between the observed (OBS) and population model-predicted (PRED) concentrations, and Fig. 1B shows the relationship between the OBS and individual model-predicted concentrations (IPRED). A good correlation in the plots was observed, suggesting that the resulting model fits the observed data well in the patients, although peak concentrations in several individuals were slightly underestimated. The diagnostic plots of the model are also shown in Fig. 2. The weighted residual values (WRES, $-2.81$, 4.72) for model-predicted concentration shown in the rectangular distribution were well acceptable. Moreover, the goodness of fit with the model had no significant difference among the patients.

The partial residual ($\eta_i$) on $CL/F$ with the key covariates was compared between the basic and resulting models (Fig. 3). The trend in the parameter estimates from the basic model (Fig. 3, left column) declined markedly in the model (Fig. 3, right column). Height and BMI were observed to closely relate to $\eta_i$, the interindividual variability for $CL/F$. Close correlations were observed between Height and Age, between Height and CBW, and between CBW and BMI (Table 5). Since Age and CBW were more closely related to a decrease in the values of the objective function ($48.85$, 55.23), Height and BMI were removed from the model.

A number of covariates are retained in the final model. As the covariates were introduced into the model, the variance of interindividual variability ($\eta_i$) for $CL/F$ and $OFV$ became smaller and smaller, and the magnitude of difference in $\eta_i$ and $OFV$ varied among the covariates. Table 6 shows the extent of each covariate-explained $\eta_i$ and $OFV$.

The population typical values of $CL/F$ or $V/F$ from the full data set and 10 individual subsets are shown in Fig. 4. The result indicated that the $CL_{F_{TV}}$ and $V/F_{TV}$ estimates from the subsets were consistent with those resulting from the full data set (mean ± S.E.). In addition, the objective function value with fixed parameter values estimated from the 10 subsets, ranging from 23,126.3 to 23,129.7, was not significantly different from that of the full data set (23,126.0, $P > 0.05$). The mean values and 95% CI for the two sets are indicated in Table 4 (the right half).

A great improvement in the predictive performance of the final model was achieved as compared to the basic one. Table 7 summarizes the precision errors from the basic and final model. The estimated average SPE (0.05) was very close to zero, and its standard deviation (0.97) was close to the expected value of 1.

The simulated steady state profiles for various situations are displayed in Fig. 5. The range of concentrations varied when the covariate value changed.

### Discussion

The aim of this study was to investigate factors that may influence the pharmacokinetics of CsA after renal transplantation. Data from clinical drug monitoring in hospitalized renal transplantation patients are sparse and random. The population method is suitable to analyze this kind of data, to accommodate flexible treatment (such as dose fluctuations), and to estimate the factors that could change the pharmacokinetics, as well as to compute the interpatient and intrapatient variability.

Either a proportional or an additive fixed-effect model can be used for the study. In the former model, variation of the pharmacokinetic parameter should increase as covariate value increases. In contrast, in the additive model, the scope of variation has no significant change with parameter value, and the upper boundary is in parallel with the lower boundary. For instance, scatter plots of $CL/F$ versus CBW indicate that the relationship between $CL/F$ and CBW was additive (Fig. 6). The similar pattern for other covariates was characterized. In addition, four patterns of regression for the correlations were compared with different analyses in $CL/F$ and CBW (linear, logarithm, power, and exponent). It was noted that no significant differences...
among the analyses (Table 8) and no change in the objective function were observed. Hence, the covariate (COVR) was incorporated into the additive linear model as shown below:

$$P_{TVi} = P_{TV} + f_{COVR} \cdot (COVR_{\text{MEDIAN}} - COVR_i) \quad (8)$$

where $P_{TVi}$ and $P_{TV}$ are $i$th individual and population’s typical value of the pharmacokinetic parameter, $COVR_{\text{MEDIAN}}$ and $COVR_i$ are the population median and the $i$th individual’s covariate value, and $f_{COVR}$ is the scaling factor for influence of covariate, respectively.

It is not surprising that the effect of body weight on CL/F of CsA was significant, which was consistent with previous reports (Rui et al., 1995; Parke and Charles, 1998, 2000; Jacobson et al., 2003). A 0.118 l/h change on CL/F would occur on every kilogram variation on CBW, which was comparable to the value (0.101–0.183 l/h) previously reported (Jacobson et al., 2003). It is common that the patients can gain body weight by 10% after transplantation.
The increase in body weight could be attributed to inappropriate food intake or decreased physical activity, as well as, possibly, high-dose administration of steroid. Therefore, monitoring the body weight during the treatment is necessary.

It has been accepted that CsA is metabolized by cytochrome P450 3A4 (CYP3A4). Thus, pharmacokinetics of CsA can be altered by either induction or inhibition of the enzyme (Yates et al., 2003). Verapamil and diltiazem have been known to be potent competitive inhibitors of CYP3A4 in the metabolism of CsA (Pichard et al., 1990; Jones et al., 1997). As reported, CsA was usually coadministered with the inhibitors to improve therapeutic potency or to reduce cost of the treatment. The studies demonstrated that CL/F of CsA in the presence of verapamil or diltiazem decreased by 30–50% as compared to that in patients with CsA alone (Masri et al., 1994; Parke and Charles, 1998). CsA is known to be metabolized by CYP3A4 and, to a lesser extent, by CYP3A5. It is also a substrate for the cell efflux transporter, P-glycoprotein. Since CYP3A4, CYP3A5, and P-glycoprotein have been shown to exist in the liver as well as in the intestine, it seems possible that the metabolism of CsA would also occur at both organ sites. Thus, it is controversial that the difference of pharmacokinetics was mainly due to temporal changes in CL/F and/or F (bioavailability). Interestingly, Preuner et al. (1998) reported that at low therapeutic CsA concentrations, diltiazem increased Cmax (maximum concentration) and areas under the curve with minor change in Ctrough. However, at high CsA concentrations, an alternative metabolic pathway was detectable, not inhibited by diltiazem (Preuner et al., 1998). Other studies also suggested that diltiazem enhances the absorption of CsA (Foradori et al., 1998). The phenomenon was similarly observed with verapamil (Tortorice et al., 1990). It has been shown that CL/F for CsA can be affected by bilirubin level (TBIL). The clearance decreased as TBIL increased. The biliary route is believed to be the major elimination pathway of CsA in a manner proportional to bile production and liver function. Previous reports indicated that dysfunction in hepatic bile excretion with high plasma TBIL correlated closely with high blood concentrations of CsA (Sun et al., 2001). Besides, the present study showed that HCT is a factor that changes CL/F, consistent with the previous report (Yee et al., 1988). This is probably due to the fact that about 50% of CsA bound to red blood cells and only free drug was clearable. The results including TBIL and HCT are in agreement with those from non-population analysis. The effect of HCT in hematopoietic stem cell transplant patients on CL/F was not evaluated due to infrequent HCT measure and alteration for red blood cell transfusion (Jacobson et al., 2003). In contrast, routine and biochemical blood examinations were performed in the present study, and frequent HCT measure at the early post-transplant stage was high, which allowed measurement for all Ctrough. Therefore, evaluation of the relationship between CL/F and TBIL/HCT was feasible.

It was reported that POD significantly altered CL/F for CsA (Rui et al., 1995; Jacobson et al., 2003). The value of CL/F declined after operation, especially within the first 3 weeks. Our results from the proposed model agreed with these reports, in that CL/F for CsA decreased by 4.35% in week 1, 8.70% in week 2, and 13.05% in week 3 after renal transplant operation. Modeling a time-related change in CL/F greatly improved goodness of fit (Fig. 7). However, attention should be paid that POD also had a great effect on oral absorption for CsA, since the altered gastrointestinal motility after operation was anticipated (Parke and Charles, 1998, 2000).

Large interindividual variability for Kc (CV% = 179) was determined by NONMEM. This is probably due to insufficient retrospective concentration data that cannot provide adequate information for analysis of the CsA absorption phase. However, since Kc values were collected from the previous study, differences of the characters between the populations from the previous study and the present study might exist. Since Neoral was used with most patients, the interindividual variability for Kc caused by different product batches of CsA can be excluded.

The population model has been defined in the present study to contain several factors involved in CL/F for CsA. Therefore, the model validation is necessary. Our internal validation analysis confirmed that the model is robust and stable based on the coincidence between NONMEM estimate and Jackknife estimate (Table 4). No significant difference was observed between the subsets and the full data set. External validation is the most stringent test of a model. It is obvious that the performance (precision and accuracy) of the final model is better than that of the basic one in terms of ME, MSE, and RMSE (Table 7). The 95% confidence interval of SPE includes zero, which indicates that the final model fits the observed concentrations well. Moreover, the S.D. of SPE (0.97) is close to 1, confirming that the contribution of predicted concentrations is in accordance with those of the observed values and that the final model is valid.

Because only one covariate value was changed for each situation, the simulated concentrations did not vary greatly. However, the demographic background and dosing regimen in the real world are more complex, and the concentrations will vary remarkably. Based on this study, special attention should be paid if a patient’s key covariate value is abnormal. Simulation with the current final model will help to treat patients individually and ensure the concentration varies within the therapeutic window.

The aim of this study was to demonstrate whether routine therapeutic drug monitoring data (C0 and C2) can be used to estimate the population parameters with NONMEM. It is popular to use C0 and C2 as markers in today’s therapeutic CsA monitoring (Ray et al., 2003; Trompeter et al., 2003). The final model proposed in this study showed its competency in predicting CsA concentrations based on the patient information.

In conclusion, a population pharmacokinetic model for CsA in renal transplant patients receiving multiple oral doses has been successfully established. The model provides a useful tool that can be employed to estimate individual CL/F, V/F, and Kc for the patients receiving CsA, and to adjust dosing regimens with covariate factors (POD, TBIL, CBW, Age, INHL, and HCT) that possibly interfere with the population pharmacokinetic parameters.

References


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