

Population Pharmacokinetics of Cyclosporine in Cardiopulmonary Transplant Recipients

Sara E. Rosenbaum,* Gautam Baheti,*‡ Andrew K. Trull,§† and Fatemeh Akhlaghi*

Abstract: A population pharmacokinetic analysis of cyclosporine (CsA) was performed, and the influence of covariates on CsA oral clearance and relative bioavailability was investigated. Data from 48 recipients of heart–lung (n = 21) or single (n = 18) or double (n = 9) lung transplant were included in the study. Patients received oral CsA as either a conventional formulation (Sandimmune™) or a micro-emulsion (Neoral™). Steady-state CsA concentrations were measured before and at approximately 2 and 6 hours after the morning dose of CsA at the end of weeks 1, 2, 3, 4, 13, 26, 39, and 52 posttransplantation. A total of 1004 CsA concentration observations were analyzed using mixed effects-modeling (NONMEM). A 1-compartment pharmacokinetic model and first-order oral absorption were used to fit the data. The absorption rate constants were fixed at 0.25 L/h for Sandimmune and 1.35 L/h for Neoral formulations. Oral clearance (CL/F) was estimated to be 22.1 L/h (95% confidence intervals [CI] 19.5–24.7 L/h). Itraconazole (ITRA), cystic fibrosis (CF), and weight (WT) were identified as significant covariates for CL/F according to the final model: $CL/F = 22.1 - 11.3 \times ITRA + 23.5 \times CF + 0.129 \times (WT - 58.7)$ L/h; where ITRA = 1 if the patient was taking concomitant itraconazole, otherwise 0; CF = 1 if the patient had cystic fibrosis, otherwise CF = 0; and WT is patient weight in kilograms. The relative oral bioavailability of Sandimmune to Neoral was 0.82. The bioavailability of both preparations increased during the first month posttransplantation. Age, gender, and type of transplant (single, double, or heart–lung) were not identified as significant covariates for CsA clearance. The population pharmacokinetic model developed identified some sources of variability in CsA pharmacokinetics; however, an appreciable degree of variability is still present in this patient population.

Key Words: cyclosporine, pharmacokinetics, lung transplant, NONMEM

(*Ther Drug Monit* 2005;27:116–122)

Received for publication August 26, 2004; accepted October 12, 2004.

From the *Department of Applied Pharmaceutical Sciences, College of Pharmacy, University of Rhode Island, Kingston Rhode Island; ‡MDS Pharma Services, Lincoln, Nebraska; and §Department of Pharmacology, Papworth Hospital, Cambridge, United Kingdom; †deceased.

In memory of the late Dr Andrew Trull, who unfortunately passed away on April 1, 2004.

Reprints: Fatemeh Akhlaghi, PhD, Applied Pharmaceutical Sciences, University of Rhode Island, 125 Fogarty Hall, 41 Lower College Road, Kingston, RI 02881 (e-mail: fatemeh@uri.edu).

Copyright © 2005 by Lippincott Williams & Wilkins

The calcineurin phosphatase inhibitor cyclosporine (CsA) is a potent immunosuppressant commonly used following organ transplantation and for the treatment of autoimmune diseases. At a cellular level CsA preferentially inhibits antigen-triggered signal transduction in T lymphocytes, thereby blunting the expression of lymphokines including IL-2 as well as antiapoptotic proteins.¹

The pharmacokinetics of CsA is characterized by a large degree of inter- and intraindividual variability and poor correlation between blood CsA concentration and the given dose.² Following oral ingestion, CsA absorption is incomplete, resulting in a low oral bioavailability.² CsA is distributed widely in various tissues and is highly bound to blood cells and plasma proteins.³ It is metabolized by isoenzymes belonging to CYP 3A4 family⁴ and is substrate for P-glycoprotein⁵; hence, it may interact with many of the inducers and inhibitors of both of these systems. CsA therefore has a narrow therapeutic index in clinical practice, and its concentration in whole blood before a dose (C_{trough}) has traditionally been monitored to aid with dosage individualization. However, the trough concentration is considered an inadequate index for monitoring, and in recent years measuring the concentration at 2 hours postdose (C-2) has been proposed as an alternative monitoring strategy.⁶

Immunosuppressive therapy in lung transplant recipients is particularly challenging. Recipients of lung or heart–lung transplants often experience repeated episodes of pulmonary rejections or infections.⁷ Unfortunately, the clinical symptoms of a lung rejection episode are similar to those of a lung infection, and the use of transbronchial biopsies to diagnose rejection is often inconclusive. Repeated injuries to the transplanted lung often result in the development of bronchiolitis obliterans syndrome (BOS), an end-stage and potentially fatal lung disease that is the major limitation to long-term survival after lung transplantation. Acute rejection episodes in the early postoperative stage have been shown to be the most significant risk factor for the development of BOS⁸ following lung and heart–lung transplant. Optimizing the immunotherapy by means of careful monitoring of immunosuppressive agents is therefore imperative to prevent acute rejection and to improve the long-term survival of these patients.

The objective of this study was to investigate the population pharmacokinetics of CsA in a cohort of cardiopulmonary transplant recipients, who participated in a randomized clinical trial of 2 different CsA formulations: Sandimmune™ and Neoral™ (Novartis Pharmaceuticals, UK).⁹ The aim was to identify the factors that elucidate the sources of variability in CsA pharmacokinetics including time

posttransplantation (TPT), cystic fibrosis (CF), CsA dosage formulation, age, gender, weight, type of transplant, and concomitant use of itraconazole.

PATIENTS AND METHODS

The population pharmacokinetics of CsA were studied using data obtained from a previously published study.⁹ The original study was a randomized, open-labeled clinical trial in lung and heart–lung transplant recipients receiving either Sandimmune or Neoral (Novartis Pharmaceuticals, UK) formulations of CsA. The study, which was conducted at Papworth Hospital, Cambridge, UK, was approved by the local Ethics Committee. In addition, approval from the Institutional Review Board (IRB) at the University of Rhode Island was obtained to reanalyze the data.

Immediately following transplantation, patients received intravenous methylprednisolone and rabbit antithymocyte globulin as induction immunosuppressive therapy, followed by maintenance triple therapy with oral CsA, azathioprine, and prednisolone. On the first day of their transplant, a dose of 50 mg of CsA (either the Sandimmune or Neoral formulation) was administered orally to the patients, and the dose increased by 50 mg at each 12-hour dose until target trough levels were achieved. Patients with cystic fibrosis (n = 8) received their daily oral CsA at 8-hour intervals rather than 12-hour intervals. Target concentrations for the trough levels were 300–400 µg/L for months 1 and 2 and 200–300 µg/L for months 3 to 12.

Abbreviated blood CsA concentration–time profiles were obtained during clinical follow-up visits at approximately weeks 1, 2, 3, 4, 13, 26, 39, and 52 posttransplantation. In general, each individual provided 3 blood samples per visit obtained at approximately 0, 2, and 6 hours after the dose. CsA concentrations in whole blood were measured in an accredited clinical laboratory by the use of a homogeneous enzyme-multiplied immunoassay technique (EMIT® 2000, Dade-Behring Diagnostic UK, Ltd; Milton Keynes, UK). The lower limit of quantification of the assay method was 50 µg/L, and the intra- and interday coefficients of variations (n = 6) were less than 10% and 21% at 500 µg/L and 50 µg/L, respectively.

Dosing history, blood CsA concentrations, and demographic and other relevant data collected over 12-month period were stored in a computer database. All data required for the present analysis were extracted and formatted for population pharmacokinetics analysis in Microsoft® Excel 2000. The concentration–time data and relevant demographic data were tabulated and checked for completeness and consistency. The pharmacokinetic analyses were performed using NONMEM (version V, level 1.1).¹⁰ In total, approximately 335 abbreviated pharmacokinetic profiles were available, providing a total of 1004 CsA concentration observations. Each abbreviated profile had a trough CsA concentration obtained before the morning dose of CsA, a concentration obtained at approximately 2 hours after the morning dose and a concentration obtained at 6 hours after the dose.

Because of the sparse nature of the data, a 1-compartment model with first-order absorption was used (ADVAN2 and TRANS2) to fit the data. The model was parameterized using the first-order absorption rate constant (k_a), oral clearance

(CL/F), and apparent volume of distribution (V/F). The relative bioavailability of CsA was also evaluated under several circumstances. Because of the limited concentration–time data in the initial period after the dose, the values for the absorption rate constants for Sandimmune and Neoral were not estimated but fixed to literature values (0.25 and 1.35 L/h, respectively).¹¹

Interindividual variability in CL/F and V/F were modeled using either an additive, proportional, or exponential error model as follows:

$$\theta_i = \theta' + \eta_{\theta_i} \text{ (additive error model)}$$

$$\theta_i = \theta' [1 + (\eta_{\theta_i})] \text{ (proportional error model)}$$

$$\theta_i = \theta' \exp(\eta_{\theta_i}) \text{ (exponential error model)}$$

where θ_i is the estimate for a pharmacokinetic parameter in the i th individual, θ' is the population mean of the pharmacokinetic parameter, and η_{θ_i} represents a random variable with a mean of zero and variance of ω^2 that distinguishes the i th individual's pharmacokinetic parameter from the population mean value predicted by the regression model.

Furthermore, a proportional error model, an additive model, and a combined additive and proportional error model were evaluated for residual variability. These models were as follows:

$$C_{ij} = C'_{ij} (1 + \varepsilon_{1ij}) \text{ (proportional model)}$$

$$C_{ij} = C'_{ij} + \varepsilon_{1ij} \text{ (additive model)}$$

$$C_{ij} = C'_{ij} (1 + \varepsilon_{1ij}) + \varepsilon_{2ij} \text{ (combined proportional and additive)}$$

where C_{ij} is the observed serum concentration of the i th individual at time j , C'_{ij} is the model's predicted serum concentration of i th individual at time j , and ε_{1ij} and ε_{2ij} are random variables with means of zero and variances σ_1^2 and σ_2^2 , respectively.

The pharmacokinetic analysis was initially conducted using the first-order (FO) estimation method in NONMEM. A base model was first developed without any covariates for the pharmacokinetic parameters. Literature ranges for the pharmacokinetic parameters of CsA were used as initial estimates. A model-building process was then employed to examine the influence of patient covariates on the pharmacokinetic parameters. The effects of the following patient covariates on CL/F were evaluated individually: age, weight, gender, cystic fibrosis, type of transplant (single-lung, double-lung, and heart and lung), time posttransplantation, and use of concomitant itraconazole. Additionally, the presence of cystic fibrosis, time posttransplantation, and formulation type (Sandimmune or Neoral) were also evaluated as potential covariates for relative bioavailability. Age and time posttransplantation were examined as continuous variables. Weight was examined as a continuous variable centered near the mean weight of the patients. Gender, formulation type, presence of cystic fibrosis, type of transplant, and the use of itraconazole as a concomitant medication were examined as categorical variables. Because of the limited concentration–time data in the initial period after

the dose, it was not possible to include a model for interindividual variability of V/F in the population. Thus, the effect of patient characteristics on this parameter was not studied.

The significance of covariates was assessed by the precision of parameter estimates, by the reduction in interpatient and residual variability, and by the change in objective function. A decrease in the minimum value of objective function of 3.841 or greater following introduction of a single covariate into the model was considered statistically significant ($P < 0.05$ with 1 degree of freedom) using the χ^2 distribution. If the 95% confidence intervals for the coefficient included the null value, the covariate was considered to be of only borderline significance and was not included in the full model. The significance of covariates was also assessed by the precision of the parameter estimate (95% confidence interval) and by the reduction in interindividual and residual variability. All the significant covariates identified above were included in the full model. A backward elimination process was then employed to identify the significant covariates for the final model using the FO method. An increase in the objective function of 7.88 or greater ($P < 0.005$) on removal of a covariate from the full model signified that the variable was significant and required for the final model. Once the final model had been developed using the FO method, the statistical component of the model was further refined. Finally, this model was then subjected to backward elimination as described above using the more rigorous first-order conditional estimate (FOCE) INTERACTION method in NONMEM. The FOCE INTERACTION method was used to allow residual error to be evaluated based on conditional parameter estimates rather than typical values.^{12,13} This approach appears to provide more accurate assessment of the significance of covariates.¹³

RESULTS

The demographic characteristics of the patients are shown in Table 1. The 48 patients were an average of 42 years old (range 19 to 66 years) and had an average weight of 58.7 kg (range 39.6–88.0 kg). The group (26 male and 22 female patients) included patients who had undergone either single-lung (18 patients), double-lung (9 patients), or both heart and lung (21 patients) transplants. Twenty-one patients received Sandimmune, and 27 patients Neoral. Eight patients had cystic fibrosis (7 male and 1 female). Eleven patients took concomitant itraconazole (400–600 mg/d) at some time during the 12-month period. These patients provided a total of 36 blood CsA concentrations during itraconazole treatment. The mean \pm SD oral prednisolone dose was 24.8 ± 19.1 mg/d at the first posttransplantation month, reducing to 8.8 ± 4.1 mg/d by 12 months posttransplantation. Further, azathioprine dose was 93 ± 38 mg/d at the first month posttransplantation and 61 ± 29 mg/d at 12 months posttransplantation.

The pharmacokinetics of CsA were modeled using a 1-compartment model with first-order absorption. As discussed earlier, the limited number of blood samples collected during the absorption phase precluded the modeling of the absorption of CsA. During the development of the base model, interindividual error for CL/F was best described using the exponential model, and a proportional error model was found to

TABLE 1. Characteristics of Study Patients (n = 48)

Characteristic	Data
Demographic data	
Gender (male/female)	26/22
Mean age (years)	42 (range 19–66)
Mean total body weight (kg)	58.7 (range 39.6–88.0)
Cystic fibrosis	8 patients
Transplant type	
Single-lung	18 patients
Double-lung	9 patients
Heart and lung	21 patients
Formulation (Sandimmune/Neoral)	21/27
Concomitant itraconazole	11 patients‡
Pharmacokinetics data	
Number of cyclosporine concentrations	1,004
Number of samples per patient (range)*	21 (3–24)
Mean \pm SD of cyclosporine dose (mg/d)†	417 ± 218

*Three blood samples were obtained during a dosing interval at approximately 0, 2, and 6 hours after dose. This was repeated for a maximum of 8 occasions over the course of the first year posttransplant.

†Non-cystic fibrosis patients received cyclosporine (25–450 mg) every 12 hours; cystic fibrosis patients received cyclosporine (100–500 mg) every 8 hours.

‡Four patients who received concomitant itraconazole were on Sandimmune, and 7 patients were on the Neoral arm of the study.

best model residual variability. The 95% confidence intervals for the estimates of interindividual variability on V/F contained the null value, and thus, interindividual variability was not modeled on this parameter.

The pharmacokinetic parameter estimates (95% CI) from the base model for CL/F and V/F were 23.1 (19.8–26.4) L/h and 202 (159–245) L, respectively. The interindividual variability in the estimate of CL/F, expressed as approximate percentage coefficient of variation (CV%), was 32.1%. Residual error was estimated to be 60.1%.

Table 2 shows the effect of the various patient characteristics when added individually as covariates for either CL/F or relative bioavailability (F_{rel}). Concomitant itraconazole, cystic fibrosis, gender, and patient weight were identified as significant covariates for CL/F. The type of transplant was found to be significant based on the change in OBJ function ($P < 0.05$). However, the 95% CI for the coefficient included 0. Thus, as described in the Methods, this was considered to be only borderline significant and not included in the full model. Patient age was not found to be a significant covariate for CL/F ($P > 0.05$). Type of formulation and cystic fibrosis were identified as significant covariates for F_{rel} ($P < 0.005$).

To probe the potential effect of time posttransplantation, the NONMEM data set was recoded so that each patient was given a different identification number for each clinic visit. Post-hoc values of CL/F were then estimated for each patient on each of the different clinic visits. Those occasions when the patient was taking concomitant itraconazole were removed from the data set. A plot of CL/F, normalized by CL/F at the first visit, against time posttransplantation indicated that CL/F decreased to $80 \pm 15\%$ of the initial value by week 4 and then recovered to $96 \pm 17\%$ by 12 months (Fig. 1).

TABLE 2. Summary of Analysis of Covariate Effect Tested on Oral Clearance or Relative Bioavailability

Covariates	Model	Δ OBJF	Θ1 (L/h)	Covariate Parameters Θ5,Θ6	IIV (%CV)
Base model	CL/F = Θ1 (OBJ = 457.0)	NA	23.1 (19.8–26.4)	NA	32.1
Itraconazole*	CL/F = Θ1 - Θ5 × ITRA	556.8	28.0 (24.4–31.6)	16.4 (12.0–20.8)	30.9
Cystic fibrosis*	CL/F = Θ1 + Θ5 × CF	282.8	21.7 (18.6–24.8)	32.7 (19.0–46.4)	23.7
Time posttransplant	CL/F = Θ1 × e ^{-Θ5 TPT}	91.0	27.5 (22.9–32.1)	0.0079 (0.001–0.17)	30.9
Gender*	CL/F = Θ1 + Θ5 × GENDER	160.9	18.3 (13.1–23.5)	10.3 (2.46–18.1)	33.2
Type of Transplant‡	TYPE = 1, CL/F = Θ1 TYPE = 2, CL/F = Θ1 + Θ5 TYPE = 3, CL/F = Θ1 + Θ6	61.6	21.5 (15.0–28.0)	Θ5 = 1.12 (-9.01–11.3) Θ6 = 11.7 (-1.00–24.4)	32.1
Weight*	TVCL/F = Θ1 + Θ5 × (WT-58.7)	92.2	23 (19.9–26.1)	0.299 (0.019–0.579)	32.1
Age	TVCL/F = Θ1 + Θ5 × Age	0	23.1 (10.3–35.9)	0	32.1
Formulation*	F _{rel} = 1*FORM + Θ5 × (1-FORM)	60	NA	0.721 (0.486–0.956)	30.0
Time posttransplant*	F _{rel} = (1 - e ^{-Θ5 TPT})	231.8	NA	0.537 (0.327–0.747)	29.9
Time posttransplant*	F _{Sandimmune} = (1 - e ^{-Θ5 TPT}) F _{Neoral} = (1 - e ^{-Θ6 TPT})	239.0	NA	Θ5: 0.456 (0.273–0.639) Θ6: 0.584 (0.335–0.833)	29.1
Cystic fibrosis	F _{rel} = 1 - Θ5 × CF	251.0	NA	0.641 (0.546–0.730)	22.9

*Denotes statistical significance, *P* < 0.05; values are mean and 95% confidence intervals.

‡Not included in the final model because estimate involved a null value in 95% CI.

Δ OBJF, decrease in the minimum objective function compared to the base model; CF, CF = 1 if patient has cystic fibrosis, otherwise 0; FORM, cyclosporine formulation; Neoral = 0, Sandimmune = 1; F_{rel}, relative bioavailability; gender, male = 1, female = 0; IIV, Interindividual variability; ITRA, 1 if patient is taking itraconazole, otherwise 0; TPT, time posttransplant in weeks; TYPE, type of transplant: TYPE = 1 for heart and lung, TYPE = 2 for single-lung, and TYPE = 3 for double-lung transplant; WT, weight in kilograms.

Previous reports on the relationship between CL/F and time posttransplantation have suggested that CsA bioavailability or clearance or both may change during the early period following transplantation.^{14,15} Thus, time posttransplantation was investigated as a covariate for both CL/F and F_{rel}. Based on the change in objective function, an exponential model for the influence of time posttransplantation was found to best fit the data (Table 2) and also found to be a significant covariate for both CL/F (CL/F = Θ1 × e^{-Θ5 × TPT}) and relative bioavailability (F_{rel} = 1 - e^{-Θ5 × TPT}) individually. Furthermore, when posttransplantation time was included as a covariate for relative bioavailability, a better fit was obtained when the 2 formulations had individual covariates (Table 2).

Probably because of the interrelationship between F_{rel} and CL/F, it was not possible to obtain model convergence

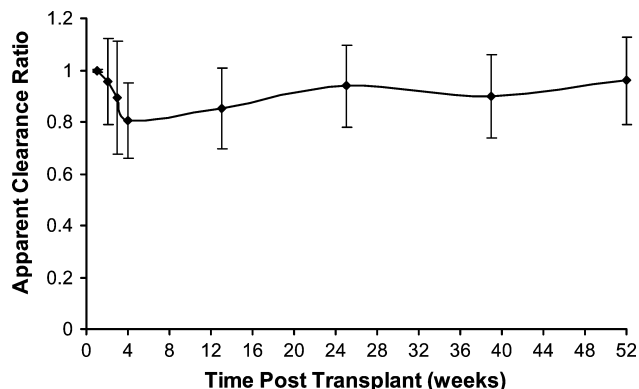


FIGURE 1. Mean ± SD of cyclosporine oral clearance normalized relative to the oral clearance at the first week posttransplantation.

when cystic fibrosis and time posttransplantation were included as covariates for both relative bioavailability and CL/F. In the full model, cystic fibrosis and time posttransplantation were modeled with the parameter (either CL/F or F_{rel}) that resulted in the larger change in the objective function when they were added individually (Table 2). Thus, in the full model, cystic fibrosis was modeled as a covariate for CL/F, and time posttransplantation for F_{rel}.

The full model containing all of the significant covariates was then subjected to backwards elimination (data not shown). As a result of this process, gender was eliminated from the model and individual parameters for Sandimmune and Neoral for the influence of time posttransplantation could no longer be justified statistically and a combined parameter for both formulations was used. Thus, the final model from the FO estimation method contained itraconazole, cystic fibrosis, and weight as covariates for CL/F, and formulation and time posttransplantation as covariates for apparent bioavailability. Once this model had been identified, the statistical component of the model was refined. As a result, a combined additive and proportional error model was found to best describe residual variability.

As a final step in the analysis, the final model obtained using the FO estimation method was subjected to backward elimination using the FOCE INTERACTION method. The statistical significance of all the covariates was confirmed using this more rigorous estimation method (data not shown). The final model parameters derived from the FOCE INTERACTION procedure are shown in Table 3. It can be seen that the modeling process resulted in a substantial reduction in the interindividual variability of CL/F and residual variability. Additionally, a plot of observed blood CsA concentrations versus predicted concentration was much closer to the line of unity for the final model than for the

TABLE 3. Comparison of Parameter Estimates for Base and Final Model

Parameter	Base Model (FO Method)	Final Model (FOCE Interaction)
CL/F (L/h)	23.1 (19.8–26.4)	22.1 (19.5–24.7)
V/F (L)	202 (159–245)	147 (130–164)
Θ5: Itraconazole	NA	11.3 (7.36–15.2)
Θ6: Cystic fibrosis	NA	23.5 (15.1–31.9)
Θ7: Weight	NA	0.129 (0.022–0.236)
Θ8: F Sandimmune	NA	0.820 (0.661–0.979)
Θ9: TPT	NA	0.886 (0.676–1.10)
IIV: CL/F (% CV)	32.1	17.1
Residual variability		
proportional (% CV)	60.1	44.0
additive	NA	76.4 μg/L

The values are reported as mean and 95% confidence intervals of the mean.

A 1-compartment model with first-order absorption was used.

Final model:

$$CL/F (L/h) = 22.1 - 11.3 \times ITRA + 23.5 \times CF + 0.129 \times (WT - 58.7).$$

$$\text{Relative bioavailability of Sandimmune} = 0.820.$$

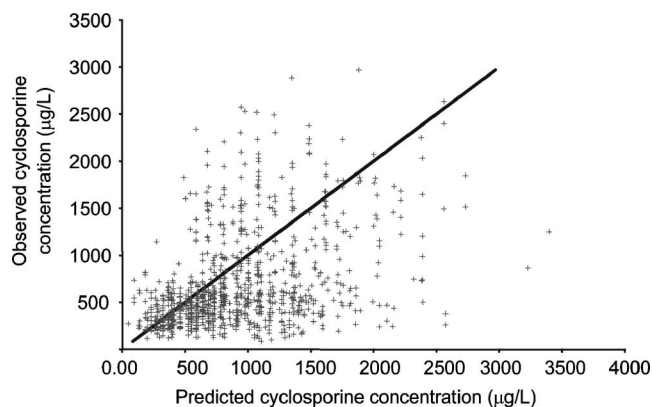
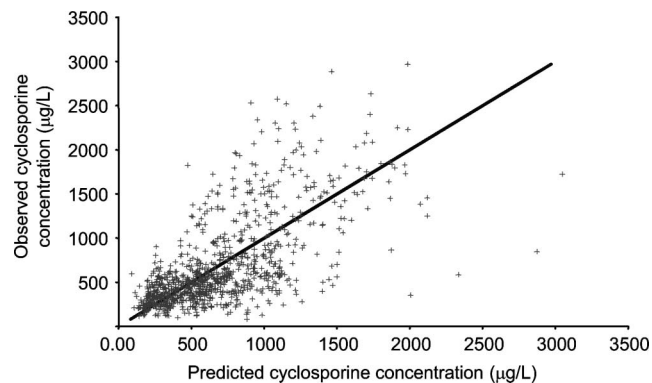
$$F = (1 - e^{-0.886 \times TPT}).$$

$$k_a \text{ fixed: Sandimmune} = 0.25/h, \text{ Neoral} = 1.35/h.$$

A proportion error model for residual error was used for the base model, and a combined additive and proportional model for the final model.

CF = 1 if patient has cystic fibrosis, otherwise 0; CL/F, oral clearance; FO, first-order method; FOCE, first-order conditional estimates; IIV, interindividual variability; an exponential model was used for IIV in CL/F in both the base and final models; ITRA = 1 if patient is taking itraconazole otherwise 0; NA, not applicable; TPT, time posttransplant in weeks; V/F, volume of distribution; WT, weight in kg.

initial base model (Figs. 2, 3). Cystic fibrosis was found to cause an increase in oral clearance values by about 108% whereas concomitant administration of itraconazole was found to decrease CL/F by about 50%. Weight was found to have a modest effect on CL/F. For example, assuming the absence of cystic fibrosis and itraconazole, the weight range in this population (39.6–88.0 kg) would be associated with a range of 19.64–25.88 L/h in CL/F. Formulation type was found to be a significant covariate for bioavailability. Sandimmune was found to have a relative bioavailability of 82.0% compared with Neoral. The rate constant for the exponential increase in

**FIGURE 2.** Model-predicted versus observed cyclosporine concentration obtained from initial model based on population parameter estimates (n = 1004). The solid line represents the line of identity.**FIGURE 3.** Model-predicted versus observed cyclosporine concentration obtained from final model based on population parameter estimates (n = 1004). The solid line represents the line of identity. One-compartment model with first-order absorption was used. The final model is given by:

$$CL/F = 22.1 - 11.3 \times ITRA + 23.5 \times CF + 0.129 \times (WT - 58.7) L/h$$

Relative bioavailability of Sandimmune = 0.820. $F_{rel} = (1 - e^{-0.886 \times TPT})$ where k_a is taken as fixed: Sandimmune = 0.25/h and Neoral = 1.35/h. An exponential model was used for interindividual variability in CL/F in both the base and final models. A proportion error model for residual error was used for the base model, and a combined additive and proportional model for the final model. Abbreviations: CF = 1 if patient has cystic fibrosis, otherwise 0; CL/F = oral clearance; F_{rel} = relative bioavailability; IIV = interindividual variability; ITRA = 1 if patient is taking itraconazole, otherwise 0; NA = not applicable; TPT = time posttransplantation in weeks; V/F = volume of distribution; WT = weight in kilograms.

F_{rel} after transplantation was found to be 0.886/wk. Thus, based on first-order kinetics, one would expect the F_{rel} to stabilize at about the fourth week posttransplantation.

Table 4 represents the mean and 95% confidence interval values of the observed and predicted CsA concentrations at times 0, 2, and 6 hours after dose. The mean predicted CsA concentrations at times 0 (C0) and 2 hours (C2) are 2.3% and 5.8% different from the observed values while the predicted concentration at 6 hours postdose (C6) is approximately 15% higher than the observed CsA concentration.

DISCUSSION

In this study we have characterized the population pharmacokinetics of conventional (Sandimmune) and the microemulsion (Neoral) formulations of CsA in lung transplant recipients over the first postoperative year. The main objective of this study was to investigate, using a population method, factors that influence the variability in the pharmacokinetic parameters, especially the apparent clearance. The only other population pharmacokinetics study of CsA in lung transplant patients¹⁶ did not explore the effects of covariates on the clearance of CsA. Therefore, our study is the first to characterize the interaction between various covariates on the apparent clearance of CsA in cardiopulmonary transplant recipients. In addition to the analysis described in this

TABLE 4. Mean and 95% Confidence Intervals of Observed and Predicted Cyclosporine Concentrations at Different Times Postdose

Time Postdose	Cyclosporine Concentration ($\mu\text{g/L}$)		
	Observed	Predicted	Residual
C0 (n = 335)	385 (361, 408)	376 (351, 402)	13 (-9, 36)
C2 (n = 335)	1054 (988, 1121)	992 (948, 1036)	62 (5, 118)
C6 (n = 334)	567 (538, 596)	664 (636, 693)	-98 (-127, -69)

C0, cyclosporine concentration before the dose; C2, cyclosporine concentration at approximately 2 hours postdose; C6, cyclosporine concentration at approximately 6 hours postdose.

manuscript, we are also investigating the association between CsA concentrations at 2 hours after the dose and clinical outcomes after cardiopulmonary transplantation, which will be published separately.

The conventional immunosuppressive regimen includes concomitant use of calcineurin inhibitors (CsA or tacrolimus), antiproliferative/antimetabolite agents (azathioprine or mycophenolic acid), and glucocorticoid (prednisolone or methylprednisolone). CsA has some similarities to the other calcineurin inhibitor (tacrolimus) in that it has a narrow therapeutic index and exhibits significant and unpredictable toxicities including nephrotoxicity,¹⁷ neurotoxicity,¹⁸ and hepatotoxicity. In a clinical setting, as a guide to dosage adjustment, total (protein-bound and free) concentration of CsA is monitored on a regular basis. However, the association between this concentration and either the clinical outcomes or the side effects is not clear; the optimal therapeutic range is not fully established,¹⁹ and the time of sampling (trough concentration versus AUC or concentration at 2 hours postdose) is subject to debate.⁶

Of the factors considered in this transplant group, cystic fibrosis was the most important patient characteristic to influence the pharmacokinetics of CsA, whereas neither patient's gender nor type of transplant had a major influence. Cystic fibrosis is a disease of the exocrine system with severe consequences on lipid absorption. Transplant recipients with cystic fibrosis absorb CsA poorly, especially from non-microemulsified formulations like Sandimmune.²⁰ The Neoral formulation, however, has been shown to provide better absorption and higher CsA concentrations in bile-deficient liver transplants²¹ or lung transplant recipients with cystic fibrosis²⁰ as compared with Sandimmune. Our final model indicates that on average the apparent clearance of CsA in transplant recipients with cystic fibrosis is 2-fold higher than the non-cystic fibrotic patients.

Comparisons between the pharmacokinetic characteristics of the oil based CsA formulation (Sandimmune) and the microemulsified formulation of CsA (Neoral) have been well documented.²² In general, administration of Neoral is associated with a faster absorption, better bioavailability and a reduced inter- and intra- individual variability in CsA exposure.²³ The relative bioavailability of Sandimmune to Neoral observed in the present study was 0.82. This value is somewhat higher than 0.74 or 0.15, the reported relative bioavailability of Sandimmune to Neoral in adult heart

transplant¹¹ or in liver transplant recipients with external biliary diversion,²¹ respectively.

The interaction between itraconazole and CsA has long been recognized.²⁴ Azole antifungal agents, including ketoconazole and itraconazole, inhibit the activity of CYP3A4 and P-glycoprotein and therefore increase the concentration of CsA reaching the systemic circulation. The concomitant use of these agents with immunosuppressive drugs in immunocompromised transplant recipients is often necessary because of repeated episodes of life-threatening fungal infections. We have found that administration of itraconazole decreases the apparent clearance of CsA by approximately 50%. This finding is consistent with the result of a conventional pharmacokinetic study of the CsA-itraconazole interaction indicating that a 48% reduction in CsA dose was required to achieve the same CsA AUC.²⁵

The relationship between CsA clearance and body weight is not clear. In a pharmacokinetic study in 10 obese uremic patients awaiting kidney transplantation, the average values for weight-adjusted clearance and volume of distribution were not significantly different from those of 35 nonobese patients.²⁶ The influence of body weight on pharmacokinetics of CsA in underweight transplant recipients, similar to the patients included in this study, has not been previously reported. The mean weight in this study was 58.7 (range 39.6–88.0) kg, and for each 10-kg deviation from the median weight the CsA apparent clearance changed by 1.2 L/h. This observation must, however, be applied with caution to other groups of transplant recipients and/or when the patient's weight is much higher than the upper end of weight range observed in this study.

The dependence of CsA clearance on time after transplantation observed in this study is in agreement with that observed in recipients of other organs.^{14,27,28} Time posttransplantation is thought to affect the bioavailability rather than clearance of CsA. In a population pharmacokinetic study in heart transplant recipients,¹⁴ the effect of postoperative day was modeled on clearance but was not found to be statistically significant.¹⁵ However, modeling postoperative day on relative bioavailability provided a superior fit for the data. In the present study, CsA apparent bioavailability increased during the first 4 weeks posttransplantation and then returned almost to baseline by 12 months. This finding is consistent with the influence of time posttransplantation in another population pharmacokinetic study in bone marrow transplant recipients.¹⁵ The mechanism for the increased bioavailability of CsA after transplantation is not clear but may be related to variation in gastrointestinal CYP3A4 and/or P-glycoprotein expression.

The most important advantage of this study was the availability of abbreviated concentration–time profiles at 8 occasions in the first year posttransplantation. This study design has enabled consideration of the effect of time posttransplantation on CsA clearance during the full course of the first postoperative year and in relation to intermittent administration of itraconazole.

One of the major limitations of this study was the fixed sampling time for CsA concentration. Population pharmacokinetic studies ideally should consist of concentration–time data obtained at randomized times across a dosing interval. The original design of this study was such that the

concentrations were obtained before dose (C₀), around 2 hours (C₂) and at 6 hours after the dose. Unfortunately, because of the paucity of information in the early period after administration, it was not possible to model the absorption characteristics of CsA, nor was it possible to evaluate potential covariates for V/F. Nevertheless, the use of fixed absorption rate constant from the literature enabled us to model the data and obtain good estimates for the oral clearance. In addition, the predicted concentrations of CsA before and at 2 hours after CsA administration were comparable to the observed values.

CONCLUSION

We have described the mixed effects modeling of CsA clearance and relative bioavailability in cardiopulmonary transplant recipients during the first postoperative year. Many of the covariates included in our model have been previously described by others; however, the model described illustrates the interplay of these important covariates in relation to the CsA clearance and bioavailability in a group of clinically difficult-to-manage transplant recipients. Although some sources of variability were identified, an appreciable degree of pharmacokinetics variability is still present in this patient population that is not accounted for by the present model. In the context of CsA therapy in lung transplant recipients, utilization of this model will allow clinicians to calculate the values of CsA apparent clearance based on patient specific demographic characteristics, CsA dosage form, or concomitant administration of itraconazole and will enable them to more objectively initiate or adjust CsA dosage. In addition, to further validate this model, a prospective clinical study is warranted in a different cohort of cardiopulmonary transplant recipients.

ACKNOWLEDGMENTS

The original clinical study was supported by an educational grant from Novartis Pharmaceuticals and was approved by the Human Ethics Committee at Papworth Hospital, Cambridge, UK. Approval from the Institutional Review Board at the University of Rhode Island was obtained to analyze the data.

REFERENCES

- Krensky AM, Storm TB, Bluestone JA. Immunomodulators: immunosuppressive agents, tolerogens and immunostimulants. In: Hardman JG, Limbird LE, Goodman Gilman A, eds. *The Pharmacological Basis of Therapeutics*. New York: McGraw-Hill, 2001:1463–1484.
- Dunn CJ, Wagstaff AJ, Perry CM, et al. Cyclosporin: an updated review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (Neoral) in organ transplantation. *Drugs*. 2001;61:1957–2016.
- Akhlaghi F, Trull AK. Distribution of cyclosporin in organ transplant recipients. *Clin Pharmacokinet*. 2002;41:615–637.
- Kronbach T, Fischer V, Meyer UA. Cyclosporine metabolism in human liver: identification of a cytochrome P-450III gene family as the major cyclosporine-metabolizing enzyme explains interactions of cyclosporine with other drugs. *Clin Pharmacol Ther*. 1988;43:630–635.
- Kelly P, Kahan BD. Review: metabolism of immunosuppressant drugs. *Curr Drug Metab*. 2002;3:275–287.
- Kahan BD, Keown P, Levy GA, et al. Therapeutic drug monitoring of immunosuppressant drugs in clinical practice. *Clin Ther*. 2002;24:330–350.
- Hausen B, Morris RE. Review of immunosuppression for lung transplantation: novel drugs, new uses for conventional immunosuppressants, and alternative strategies. *Clin Chest Med*. 1997;18:353–366.
- Sharples LD, McNeil K, Stewart S, et al. Risk factors for bronchiolitis obliterans: a systematic review of recent publications. *J Heart Lung Transplant*. 2002;21:271–281.
- Trull A, Steel L, Sharples L, et al. Randomised, trough blood cyclosporin concentration-controlled trial to compare the pharmacodynamics of sandimmun and neoral in de novo lung transplant recipients. *Ther Drug Monit*. 1999;21:17–26.
- Beal SL, Sheiner LB, eds. *NONMEM User's Guide*. San Francisco: NONMEM Project Group, University of California, 1992.
- Akhlaghi F, Keogh AM, McLachlan AJ, et al. Pharmacokinetics of cyclosporine in heart transplant recipients receiving metabolic inhibitors. *J Heart Lung Transplant*. 2001;20:431–438.
- Karlsson MO, Jonsson EN, Wiltse CG, et al. Assumption testing in population pharmacokinetic models: illustrated with an analysis of moxonidine data from congestive heart failure patients. *J Pharmacokin Biopharm*. 1998;26:207–246.
- Wahlby U, Jonsson EN, Karlsson MO. Assessment of actual significance levels for covariate effects in NONMEM. *J Pharmacokin Pharmacodyn*. 2001;28:231–252.
- Parke J, Charles BG. NONMEM Population Pharmacokinetics modeling of orally administered cyclosporine from routine drug monitoring data after heart transplantation. *Ther Drug Monit*. 1998;20:284–293.
- Jacobson PA, Ng J, Green KG, et al. Posttransplant day significantly influences pharmacokinetics of cyclosporine after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2003;9:304–311.
- Rousseau A, Monchaud C, Debord J, et al. Bayesian forecasting of oral cyclosporin pharmacokinetics in stable lung transplant recipients with and without cystic fibrosis. *Ther Drug Monit*. 2003;25:28–35.
- Rezzani R, Angoscini P, Borsani E, et al. Cyclosporine A-induced toxicity in two renal cell culture models (LLC-PK1 and MDCK). *Histochem J*. 2002;34:27–33.
- Bechstein WO. Neurotoxicity of calcineurin inhibitors: impact and clinical management. *Transpl Int*. 2000;13:313–326.
- Filler G, Mai I, Filler S, et al. Abbreviated cyclosporine AUCs on Neoral—the search continues! *Pediatr Nephrol*. 1999;13:98–102.
- Tan KK, Trull AK, Uttridge JA, et al. Relative bioavailability of cyclosporin from conventional and microemulsion formulations in heart-lung transplant candidates with cystic fibrosis. *Eur J Clin Pharmacol*. 1995;48:285–289.
- Trull AK, Tan KK, Tan L, et al. Absorption of cyclosporin from conventional and new microemulsion oral formulations in liver transplant recipients with external biliary diversion. *Br J Clin Pharmacol*. 1995;39:627–631.
- Kovarik JM, Mueller EA, Niese D. Clinical development of a cyclosporine microemulsion in transplantation. *Ther Drug Monit*. 1996;18:429–434.
- Kovarik JM, Mueller EA, van Bree JB, et al. Reduced inter- and intraindividual variability in cyclosporine pharmacokinetics from a microemulsion formulation. *J Pharm Sci*. 1994;83:444–446.
- Kwan JT, Foxall PJ, Davidson DG, et al. Interaction of cyclosporin and itraconazole. *Lancet*. 1987;2:282.
- Florea NR, Capitano B, Nightingale CH, et al. Beneficial pharmacokinetic interaction between cyclosporine and itraconazole in renal transplant recipients. *Transplant Proc*. 2003;35:2873–2877.
- Flechner SM, Kolbeinson ME, Tam J, et al. The impact of body weight on cyclosporine pharmacokinetics in renal transplant recipients. *Transplantation*. 1989;47:806–810.
- Anderson JE, Munday AS, Kelman AW, et al. Evaluation of a Bayesian approach to the pharmacokinetic interpretation of cyclosporin concentrations in renal allograft recipients. *Ther Drug Monit*. 1994;16:160–165.
- Charpiat B, Falconi I, Breant V, et al. A population pharmacokinetic model of cyclosporine in the early postoperative phase in patients with liver transplants, and its predictive performance with Bayesian fitting. *Ther Drug Monit*. 1996;20:158–164.