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Factors affecting oral cyclosporin disposition after heart transplantation: bootstrap validation of a population pharmacokinetic model

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Abstract *Objective*: To determine factors affecting the population pharmacokinetics of oral cyclosporin (CsA) in cardiac allograft recipients during the first 3 weeks after surgery.
Methods: Data were obtained from routine trough monitoring and from two extra samples drawn during a dosing interval on a randomly selected day. Whole blood CsA concentrations were assayed using high-performance liquid chromatography (HPLC). Approximately equal numbers of patients were prescribed Sandimmun (SAN) or Neoral (NEO) CsA formulations. Parameter values of a one-compartment kinetic model with first-order absorption and elimination were sought together with the inter-patient and intra-patient variances using the NONMEM program.
Results: Improved fits resulted from using the following expression in the model to adjust apparent bioavailability as a function of post-operative day (POD): $f = 0.2 + 10 \times \mathrm{ABS}\ (\mathrm{POD}-5)/[(\mathrm{POD} + 7) \times 60]$. The CsA clearance (CL/f) was found to be influenced by current body weight (WT). There was an absorption lag time of about 35 min with SAN, but zero lag time with NEO. Oral bioavailability (f) was increased by about 35% with concomitant diltiazem and about 18% with NEO. The CL/f was10% higher during the daytime than at night. The final pharmacokinetic model was validated using 200 bootstrap samples of the original data.
Conclusions: Using a validated population modelling approach, it was found that a number of factors influence the pharmacokinetics of CsA during the early post-operative period in cardiac transplant patients. These influences affecting oral bioavailability and clearance may need to be taken into account for maintaining appropriate concentrations of CsA in the bloodstream.

Key words Cyclosporin · Heart transplantation · Population pharmacokinetics

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Introduction

Oral administration of cyclosporin (CsA) in transplant patients is associated with marked intra- and inter-individual variability in blood concentrations [1]. Much of this variability has been attributed to inconsistent bioavailability [2]. Accordingly, a self-emulsifying formulation (Neoral, NEO) was developed with the goal of providing enhanced and more reliable absorption of the drug [3, 4], although this was not always achieved [5]. In a previous study, we reported the pharmacokinetics of orally administered Sandimmun (SAN) from retrospective routine monitoring data gathered over several months after cardiac transplant surgery [6]. Although the amount of data was limited, it was noted that there was an apparent reduction in CsA oral bioavailability that was most pronounced at about 7 days after patients received cardiac grafts. As with other transplant patients, it is particularly important to maintain stable concentrations of CsA in the therapeutic range for the first few weeks after cardiac surgery when graft rejection is most likely to occur.

The present study extended this work and focused on factors that may influence the pharmacokinetics and bioavailability of CsA in the early period after cardiac transplantation. To do this, routine CsA monitoring data were combined with concentration data from samples drawn in the absorption phase from patients taking two formulations of oral CsA during a 3-week

period after heart transplant. Because there were only 46 patients in the study, an independent validation of the population pharmacokinetic model could not be determined; therefore, the accuracy and robustness of the model was assessed using a novel bootstrap procedure which we describe herein.

Patients and methods

Drug monitoring data were obtained from 46 cardiac transplant recipients at The Prince Charles Hospital (Brisbane, Queensland, Australia). Characteristics of the study patients are summarised in Table 1. All patients were receiving oral "triple therapy" (i.e. CsA, azathioprine and prednisone); 20 patients received SAN and 26 received NEO formulations of CsA. No patients received CsA parenterally. Information gathered included date, time, post-operative day (POD), formulation of CsA, dose of CsA, age, height, current WT, liver function tests (total bilirubin, alkaline phosphatase, aspartate amino-transferase), serum creatinine concentration and concurrent medication. Wherever possible the data were checked thoroughly for accuracy, including testimonies from randomly selected patients.

Prior written approval of the protocol was received from the ethics committees of The Prince Charles Hospital and The University of Queensland. The study was conducted in accordance with the requirements of the Declaration of Helsinki (and subsequent amendments) and the National Health and Medical Research Council (Australia) Guidelines for Good Clinical Practice.

Blood sampling and CsA analysis

Trough (pre-dose) blood samples were drawn just prior to the morning dose of CsA (~0800 hours). In addition, on a randomly selected day within the first 21 days following transplantation, each patient was asked to provide a pre-dose (trough) blood sample, then two post-dose samples during the day at pre-randomised times. All blood samples were drawn into tubes containing ethylene diamine tetraacetic acid and stored at −20 °C. Concentrations of CsA in whole blood were assayed using a specific, reverse-phase high-performance liquid chromatography (HPLC) method [7], which has a variability (CV%) of less than 8% and an accuracy of greater than 94%.

Population pharmacokinetic model building

CsA blood concentration–time data were modelled using the NONMEM program (version 4.2) [8] on a one-compartment structural model with first-order absorption and elimination. The procedure for the screening and selection of covariates was performed as described previously [6, 9]. The variance model of the inter-subject variability and intra-subject variability was described using exponential and slope-intercept error models, respectively [8].

Table 1 Characteristics of patients in the study. Results are presented as the number or mean (range)

Characteristic	Value
Gender (male/female)	40/6
Age (years)	52 (18–67)
Height (cm)	171 (153–192)
Weight (kg)	78 (51–113)
CsA	
Dose (mg per 12 h)	217 (50–525)
Concentration (ng ml^{-1})	241 (15–1701)
Number of samples	489
Median samples per patient	11
Concomitant diltiazem:	
Always	5
Never	17
Sometimes	24
Post-operative starting day	6 (2–16)

Bootstrap validation

The accuracy and robustness of the final population model (Table 2, Table 3) were assessed using a bootstrap method [10]. The bootstrap algorithm has been a feature of some statistical packages but, to our knowledge, has been used only recently by us and others for evaluating mixed-effects population models [11, 12]. In the present context, the bootstrap concept may be explained briefly as follows. Small numbers of subjects with few data points per patient may compromise both the building and the testing of population models. Assessment of model accuracy using a small validation data sample may lead to rejection of a 'valid' model, e.g. a covariate that would otherwise attain significance in the model could be missing from the data set reserved for model validation. Moreover, there could be undue influence by a small number of patients whose responses are atypical of the rest of the cohort. A bootstrap involves repeated random sampling, with replacement, of the original data set to produce another data set of the same size as the original but with a different combination of subjects (and their data). For example, there may be three occurrences of patient number 1, none for patients 2–5, four for patient 6 and so on. As the number of bootstrap samples approaches infinity, the standard deviations of the parameters approach the 'true' (but unknown) standard deviation; in practice, the bootstrap resampling is usually repeated 200 times. Model building is then performed and the nature of the structural model (e.g. type and number of structural parameters) and the values of the parameters are compared with those obtained from the original population model. An appreciable discrepancy between the parameter values estimated from the original data and the estimated bootstrap mean values may be cause to have reduced confidence in the model. The entire procedure was performed in an automated fashion using DOS batch files, Microsoft Excel routines and Awk scripts, in conjunction with NONMEM as we have described previously [12]. The bias (expressed as the mean prediction error of observed and model-predicted concentrations) and precision (root mean square prediction error) from the final population model were compared with the mean bias and mean precision obtained from the 200 bootstrap analyses.

Results

During early screening of covariates, NONMEM had difficulty estimating the inter-patient variance about V (apparent volume of distribution) unless the off-diagonal elements of the Ω matrix (i.e. covariances of η_1 , η_2 and η_3) were modelled. This resulted in more reasonable estimates of the variability in V, together with a lowering of the objective function value (Obj) for the base model from 4902 to 4856.

Table 2 Estimation of population variability. *CL/f* clearance ($l\ h^{-1}$); *CV* coefficient of variation

Source of variability	CV%
Inter-individual variability in CL/f	19.7
Inter-individual variability in V/f	53.9
Inter-individual variability in k_a	194
Intra-individual (residual) variability	35.4

Table 3 Influence of covariates on pharmacokinetics of cyclosporin at initial screening. *Obj* objective function value; *f* oral bioavailability (percent of dose systemically absorbed); *CL/f* clearance (l h^{-1}); t_l absorption lag time (h); *WT* current body weight (kg); *HT* height (cm); *BIL* total serum bilirubin concentration (mmol l^{-1}, 1 if concentration <15, else BIL = 0); *DIL* diltiazem administration, (1 for concomitant diltiazem administration, else DIL = 0); *POD* post-operative day, (1 if post-operative day >5, else POD = 0); *NOC* diurnal effect, (1 for samples collected before morning dose, else NOC = 0); *FOR* formulation (Neoral = 1, Sandimmun = 0)

Model	Structure	Obj	Change in Obj compared with model #1	Explanation
1	$CL/f = \theta_1$	4856	Base model	
2	$CL/f = \theta_1 + \theta_2 \cdot WT$	4833	−22.98	WT affects CL/f
3	$CL/f = \theta_1 \cdot HT$	4836	−20.58	HT affects CL/f
4	$CL/f = \theta_1 \cdot DIL + \theta_2 \cdot (1-DIL)$	4834	−22.38	DIL affects C/f L
5	$CL/f = \theta_1 \cdot BIL + \theta_2 \cdot (1-BIL)$	4847	−9.05	BIL affects CL/f
6	$CL/f = \theta_1 \cdot POD + \theta_2 \cdot (1-POD)$	4824	−30.74	POD affects CL/f
7	$CL/f = \theta_1 \cdot NOC + \theta_2 \cdot (1-NOC)$	4854	−1.74	NOC on CL/f
8	$f = \theta_1 \cdot DIL + \theta_2 \cdot (1-DIL)$	4832	−24.48	DIL affects f
9	$f = 0.2 + 10(POD-5)\ (POD + 7) \cdot 60$	4832	−63.09	Post-operative time affects f
10	$t_l = \theta_1 \cdot FOR + \theta_2 \cdot (1-FOR)$	4814	−41.71	FOR affects t_l
11	$f = \theta_1 \cdot FOR + \theta_2 \cdot (1-FOR)$	4846	−9.274	FOR affects f

For the base model, plots of the average residual concentration (observed concentration minus model-predicted concentration) as a percentage of the observed concentration for each patient indicated an over-prediction in concentration that reached a maximum at POD 5, but then gradually recovered until observed and predicted concentrations were in reasonably good agreement by POD 18. This over-prediction was independent of CsA formulation.

To correct for this apparent temporal effect on bioavailability, various mathematical formulae were screened, including 2nd- and 3rd-order polynomials. However, the best fit to the data was obtained when the empirical expression, $f = 0.2 + 10 \times ABS\ (POD-5)/([POD + 7] \times 60)$, was used to adjust the apparent oral bioavailability as a function of POD. The Obj dropped markedly and a scatterplot of average percentage residual concentration on POD showed much improved agreement between observed and predicted concentrations.

Individual screening of potential covariates (Table 3) indicated that CL/f decreased with either concomitant diltiazem administration or total serum bilirubin concentration greater than 15 M, and was influenced by WT, current height (HT) and POD greater than 5 days. Concomitant administration of diltiazem increased f, as did changing from SAN to the microemulsion formulation, NEO. However, this interaction with diltiazem might indicate decreased CL, increased f or changes in both. The inhibition of CYP3A is known to be significant in the intestinal tract [13–15]. If the presently observed data were a consequence of the inhibition of intestinal wall metabolism (i.e. increased f), then CL and V would appear to change in the same direction in order to preserve a constant elimination half-life ($t_{1/2}$). If inhibition was solely in the liver, then f and V would remain unchanged, whereas CL would decrease (and $t_{1/2}$ would increase). If both systemic CL and f were affected, the result would be intermediate between these two extremes. Modelling of each possibility independently resulted in a decrease in the Obj of 22 for the effect on CL alone, and a decrease of 24 for an effect on f alone; the inclusion of both possibilities resulted in a fall in Obj of 45. In the latter case, concomitant diltiazem administration appeared to increase f by 40% with only small changes in CL and V (+2.5% and −6%, respectively). In the final model, only an effect of diltiazem on f was notable, and the simultaneous modelling of an influence of diltiazem administration on both f and CL produced no further change in the Obj. A plot of weighted residual (WRES) versus model-predicted CsA concentration (PRED) showed tighter packing around the WRES axis for an effect of diltiazem on f than for an effect on CL. Collectively, these findings indicated that concomitant diltiazem increased the f of CsA by about one-third without a major effect on systemic CL. A t_l could be fitted to the SAN data, but not for NEO data. The population value for k_a was not influenced by any covariate.

In the final structural model, the Obj value decreased when WT was included as a coefficient for CL/f and POD, while concomitant diltiazem administration and

Table 4 Mean (SEM) values of coefficients in the final structural population model. *CL/f* clearance (l h^{-1}); *WT* current body weight (kg); *f* oral bioavailability (percentage of dose systemically absorbed)

Influence of current body weight on clearance (l h^{-1})	
$CL/f = \theta_1 + \theta_2 WT$	= 4.7(1.1) + 0.0818(0.017)WT
Absorption rate constant in all patients (h^{-1})	
$k_a = \theta_3$	= 1.25(0.179)
Fractional increase in daytime clearance (l h^{-1})	
$CL/f = CL/f\theta_4$	= CL/f 1.10(0.018)
Fractional increase in bioavailability for Neoral administration	
$f = f(1.0 + \theta_5)$	= f[1.0 + 0.184(0.069)]
Fractional increase in bioavailability with concomitant diltiazem administration	
$f = f\theta_6$	= f1.35(0.0634)
Volume of distribution in all patients (l)	
$V/f = \theta_7$	= 77.4(11.3)

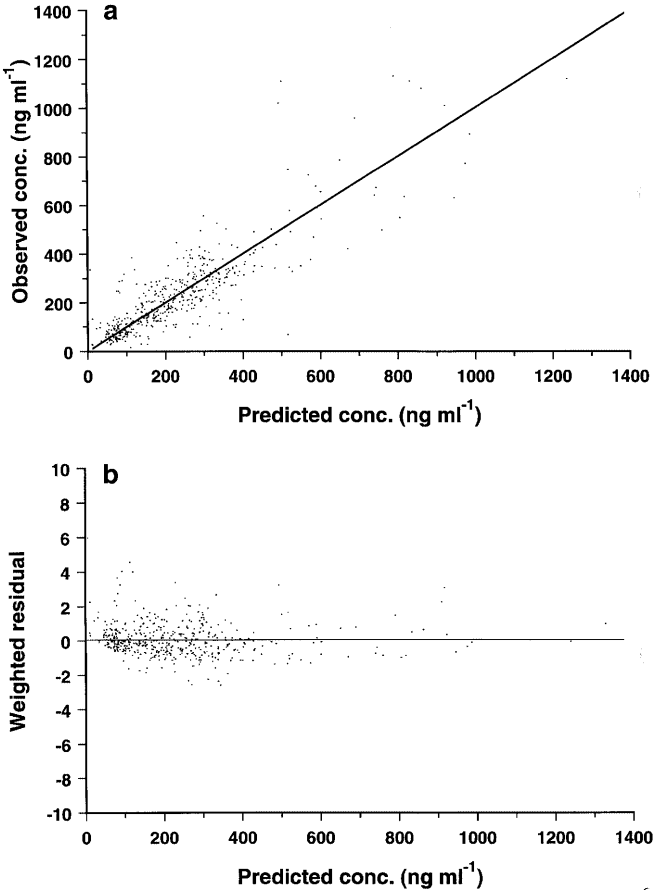

Fig. 1 Final model. *Scatterplot* of observed versus predicted cyclosporin (CsA) concentration (a) and weighted residual versus model-predicted CsA concentration (b)

CsA formulation POD were included as coefficients for f. However, whilst providing the lowest value of Obj, this model gave very high coefficients of variation (CV) for the inter-individual variances in CL/f, V/f and k_a. When a diurnal influence on CL/f was included, the CVs of the estimated values for the ηs decreased substantially, negative correlations disappeared and the bias between observed and model-predicted concentrations decreased from 16.7% to 1.40%.

The values of the structural model parameters are summarised (Table 4). Scatterplots of observed versus model-predicted CsA concentration for the final model appear in Fig. 1A. A plot of weighted residuals versus population model-predicted concentration showed a symmetrical distribution about the null ordinate (Fig. 1B). The average observed-predicted concentration bias was 1.4 $\mu g\ l^{-1}$, which represents 9.36%, 0.58% and 0.083% of the minimum (15 $\mu g\ l^{-1}$), average (241 $\mu g\ l^{-1}$) and maximum (1701 $\mu g\ l^{-1}$) CsA concentrations, respectively. The root mean square error was 109 $\mu g\ l^{-1}$. A one-compartment model was appropriate as seen by a plot of CsA concentration, normalised to a 200-mg dose, versus post-dose time within the dosing interval when the two extra samples were drawn (Fig. 2).

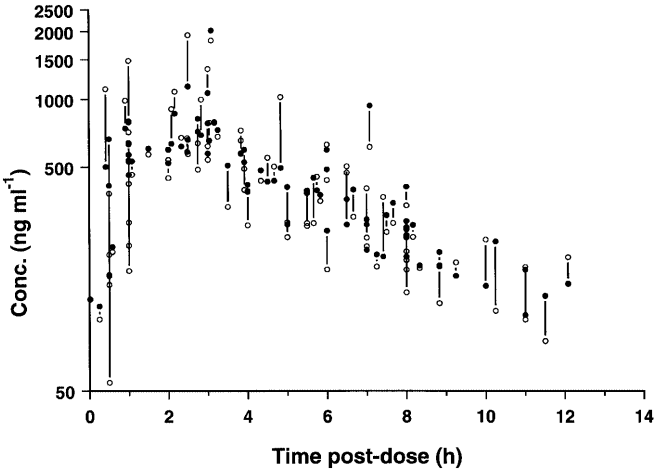

Fig. 2 Pairs of model-predicted (●) and observed (○) cyclosporin (CsA) concentrations (normalised for 200-mg dose) during the dosing interval in which two extra post-dose samples were drawn

A plot of NONMEM-calculated post-hoc estimates of CL/f for individual patients versus WT is shown (Fig. 3).

The typical CL/f for a 70-kg patient was 10.4 $l\ h^{-1}$ during the night and 11.5 $l\ h^{-1}$ during the daytime. The mean values of $t_{1/2}$ for day and night were 5.14 h and 5.66 h, respectively. The mean V/f over all patients was 77.4 l. The mean k_a was 1.25 h^{-1}, and the absorption half-life was 33 min. The mean t_l was estimated to be about 35 min for SAN and 0 min for NEO. The average f increased by about 18% when taking NEO, and increased by about 35% for patients who were taking diltiazem.

The inter-patient variabilities (CV%) in CL/f, V/f and k_a were 19.7%, 53.9% and 194%, respectively. The intra-patient variability was 373%, 35.4% and 33.2% at

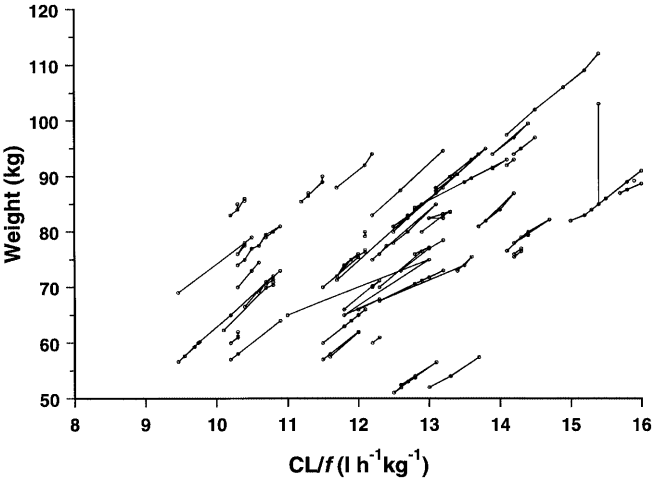

Fig. 3 Plot of posterior Bayesian estimates of cyclosporin (CsA) clearance (CL/f) versus body weight (WT). *Points* pertaining to individual patients are connected

the minimum, average and maximum CsA concentrations, respectively (Table 3). Attempts to model the inter-patient variability for the effects of diltiazem and NEO on f, NEO on t_l and diurnal variation on CL were unsuccessful. In each case, if extra ηs were included without estimating the covariance between them, their values were vanishingly small coupled with very large standard errors; however, modelling the covariances then caused premature termination due to rounding errors. This result was most likely due to the relatively small number of study subjects in each category. For the intra-patient variability, the application of an additive error model combined with a constant coefficient of variation model gave the best fit.

Bootstrap validation

The final model obtained from application of the bootstrap analysis was structurally and statistically identical with the final model developed using the 46 study patients. The parameter values obtained from the bootstrapping (average of 200 runs) and the single set of values from the original data set are shown (Table 5). When observed and model-predicted CsA concentrations were compared, both the average bias and precision estimated from the bootstrap analysis agreed favourably with the bias and precision values pertaining to the final population model (Table 5). Together with the reasonably close agreement between corresponding pairs of bootstrap and final model parameters, this indicated that the accuracy of the population pharmacokinetic model was acceptable.

Table 5 Bootstrap validation of structural and variance parameters in final model

Parameter	Final model[a,b]	Bootstrap[c]	Difference (%)[d]
Structural model[a]			
θ_1	4.70	5.00	+6.4
θ_2	0.0818	0.0890	+8.8
θ_3	1.25	1.34	+7.2
θ_4	1.10	1.09	−0.91
θ_5	0.184	0.177	−3.8
θ_6	1.35	1.32	−1.5
θ_7	77.4	81.7	+5.5
Variance model[b]			
$\omega^2_{CL/f}$	0.0389	0.0426	+9.5
$\omega^2_{V/f}$	0.291	0.307	+5.5
ω^2_{ka}	3.76	4.26	+13
$\sigma_1{}^2$	0.0169	0.0207	+22
$\sigma_2{}^2$	2920	2871	−1.68
Relative performance			
Bias	1.40	1.42	+1.4
Precision	109	111	+1.8

[a] See Table 4
[b] See Table 2
[c] Mean of 200 bootstrap analyses
[d] (Bootstrap value–final model value)/final model value 100%

Discussion

The study aimed to investigate factors that may influence the pharmacokinetics of CsA in the early period after heart transplantation. Since about equal numbers received SAN and the newer microemulsion, NEO, this also afforded an opportunity to assess the comparative bioavailability of the two formulations. Clinical studies of seriously ill patients from which there are only sparse observational responses to a drug are suited to analysis via a population approach that can accommodate unbalanced and unstructured treatments (e.g. alterations in dose and dosing interval), assess factors that can modify the pharmacokinetics and estimate the inter-individual and intra-individual variabilities.

There was a marked over-prediction of CsA blood concentrations that reached a maximum at POD 5, but then the over-prediction diminished gradually until POD 18 when there was good agreement between observed and predicted concentrations. This trend could have been due to temporal changes in CL, f or both. Modelling a time-related change in CL produced no improvement in the fit of the data, which suggested that CL was stable over time after transplantation. In general agreement with the result from one of our previous studies [6], superior fits to the data were obtained when a discrete, mathematical expression was used to adjust average oral bioavailability as a function of POD. This empirical function used was derived by trial and error and had no readily identifiable physiological basis. Most likely, it reflected the combined contribution of a number of factors, including cholestasis [16], gastrointestinal complications of surgery such as paralytic ileus and intestinal ischaemia [17] and continuous infusion of adrenaline, noradrenaline, dobutamine, dopamine and isoprenaline, separately or in various combinations during the first 3–4 days after surgery. Attempts to model a temporal change in k_a were unsuccessful, possibly because there was only one blood sample taken over the expected time frame for the absorptive phase and the number of patients was relatively small, so that it is unknown whether the above factors affected rate as well as extent of absorption. Nonetheless, the typical population absorption half-life value (obtained from the expression, $0.693/k_a$) was 33 min, indicating that the rate of CsA absorption from the intestinal tract was quite rapid. Although k_a was estimated with good precision there was very high inter-patient variability that may reflect high variability in intestinal cytochrome isoenzyme activity or variation introduced by a number of the post-operative effects on gut motility described above.

Diltiazem was screened as a potential modifier of CL/f, since it reportedly decreases the metabolism of CsA via inhibition of CYP3A [18] and since more than one-half of our patients had taken diltiazem with CsA. Presently, the administration of diltiazem resulted in a decrease in CL/f of CsA that, despite the absence of any intravenous data, was attributed mostly to reduction in

first-pass metabolism in the gut wall rather than reduction in systemic clearance in the liver [13–15]. There was a lag time (0.5 h) following administration of the SAN formulation, but this was not supported by the NEO data. These results were considered feasible since the SAN formulation must undergo emulsification in the gut before absorption. A previous study also described both a lag time from SAN (value not stated) but not NEO and a model involving first-pass metabolism in the gut which provided the best fit to the blood CsA concentration data [19]. Although it was not possible to determine absolute bioavailability from our oral data, NONMEM can nonetheless can be useful to estimate the relative f between products [20, 21]. Presently, the f of NEO was 18% greater than that of SAN. Others reported that the absolute f from SAN was 10–57%, and 35–94% from NEO [22], while in de novo cardiac transplant patients NEO administration resulted in a 35% increase in dose-normalised areas under the curve, compared with SAN [23]. Very recently it was reported that in renal transplant recipients NEO bioavailability was 22% greater than that of SAN [24]; these trends agreed generally with our results. While enhanced CsA bioavailability may provide better systemic absorption and, therefore, cheaper treatment, the switching of patients between formulations may result in different adverse-effect profiles. For example, after 24 months of treatment with NEO, only 10% of patients were free of gingival hyperplasia [25], compared with 78% for SAN [26].

The study protocol also offered an opportunity to examine whether there were diurnal changes in CL/f, since the extra pre-dose (morning) blood samples contained data reflecting the influence of the night-time CL/f, whilst at least one of the two extra blood samples subsequently drawn during the day were due to CL/f operating on the daytime dose. The finding of a slightly higher daytime CL/f generally agreed with those of Canafax and co-workers [27], who used a non-population analysis and found higher trough CsA concentrations and higher areas-under-the-curve following an evening dose than with the daytime dose. The presently observed difference between day and night clearance probably reflects reduced nocturnal hepatic metabolism [27, 28], perhaps via reduced cardiac output, but it is slight and would not warrant any change in day and night dosage based on this factor alone.

The unexplained (intra-individual) variability between observed and model-predicted CsA levels was reasonably high. Sources of such variability encompass assay variation, inaccurate sampling and dosing times and the 'wrong' pharmacokinetic model. With regard to the latter, CsA concentration data in patients from whom many samples are drawn in a dosing interval, especially after intravenous administration, may support multi-compartment kinetic models [29]. However, in the present study, each patient provided only very limited data that collectively could support only a one-compartment model. Variability in timed events is more likely to be a major contributor to this source of variability, especially when patients are unsupervised after hospital discharge. Inaccurate recording of doses in the clinic is well known [30] and, consequently, can introduce marked variability in any pharmacokinetic analysis [31].

In healthy subjects, cardiac transplant patients and children with severe cardiac disease prior to transplantation, the average V was three- to fivefold smaller than in renal and liver transplant recipients [32]. The reason for this difference is unknown, but apparently is unrelated to differences in the haematocrit. The V in the cardiac transplant patients in that study ranged from $0.9\ l\ kg^{-1}$ to $1.3\ l\ kg^{-1}$, which compared favourably with the current study value of $0.992\ l\ kg^{-1}$ (obtained by normalising the population average value of 77.4 l by average WT of 78 kg). The relatively low V of $1.3\ l\ kg^{-1}$ reported from that study, and found in the present study, most likely contributed to the comparatively short $t_{1/2}$.

The overall CL/f of $0.142\ l\ h^{-1}\ kg^{-1}$ also agreed with that reported in heart transplant recipients whose data were analysed using a traditional (non-population) approach [32]. WT was a positive influence on CL/f; in many patients WT increased in the early post-operative days and then reduced gradually as patients recovered and attained stable weights approximately 2–3 weeks following transplantation.

The final population model contained a number of factors that influenced either clearance or bioavailability; therefore, it was important to determine whether this model was sufficiently accurate and robust for predictive purposes in therapeutics. Because the number of patients was not large, this precluded the a priori subdivision of the data into an index group (for model development) and a predictive-performance set (for independent comparison). An alternative method, the bootstrap, uses all the data in an a posteriori analysis but, until recently [11, 12], this approach has received little application in evaluating mixed-effects models. Presently, the bootstrap results indicated that the 'best' model was identical in structure to that obtained with the original data set, and that all structural and variance parameter values obtained with the original data agreed reasonably well with their bootstrap mean values.

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