Population pharmacokinetic analysis of mycophenolic acid in renal transplant recipients following oral administration of mycophenolate mofetil

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Aim To develop a population pharmacokinetic model for mycophenolic acid in adult kidney transplant recipients, quantifying average population pharmacokinetic parameter values, and between- and within-subject variability and to evaluate the influence of covariates on the pharmacokinetic variability.

Methods Pharmacokinetic data for mycophenolic acid and covariate information were previously available from 22 patients who underwent kidney transplantation at the Princess Alexandra Hospital. All patients received mycophenolate mofetil 1 g orally twice daily. A total of 557 concentration–time points were available. Data were analysed using the first-order method in NONMEM (version 5 level 1.1) using the G77 FORTRAN compiler.

Results The best base model was a two-compartment model with a lag time (apparent oral clearance was 27 l h^{-1}, and apparent volume of the central compartment 98 l). There was visual evidence of complex absorption and time-dependent clearance processes, but they could not be successfully modelled in this study. Weight was investigated as a covariate, but no significant relationship was determined.

Conclusions The complexity in determining the pharmacokinetics of mycophenolic acid is currently underestimated. More complex pharmacokinetic models, though not supported by the limited data collected for this study, may prove useful in the future. The large between-subject and between-occasion variability and the possibility of nonlinear processes associated with the pharmacokinetics of mycophenolic acid raise questions about the value of the use of therapeutic monitoring and limited sampling strategies.

Keywords: pharmacokinetics, population analysis, mycophenolate

Introduction

Mycophenolate mofetil (MMF) is an immunosuppressant that can be used alone, or in combination with corticosteroids and calcineurin inhibitors for the prevention of acute renal allograft rejection [1, 2]. The efficacy of MMF has been clearly demonstrated in large, randomized, double-blinded clinical trials [3–5]. MMF, in combination with cyclosporin and prednisone, has been shown to reduce the rate and severity of acute renal allograft rejection and increase renal allograft survival at 3 years when compared with azathioprine or placebo [6]. Common side-effects of MMF are mainly due to local irritation in the gastrointestinal tract (e.g. diarrhoea and stomach discomfort) and generally resolve with dose reduction [1, 7].

MMF is an ester prodrug of its active metabolite mycophenolic acid (MPA). MPA is a noncompetitive inhibitor of inosine monophospnate dehydrogenase, a rate limiting enzyme involved in the pathway on purine synthesis [8]. T- and B-lymphocytes are mainly dependent on the *de novo* synthesis of purine for proliferation.

MMF is absorbed and metabolized rapidly by plasma esterase to MPA. MPA is further metabolized by glucuronyl transferase in the liver to mycophenolic acid glucuronide (MPAG), which is pharmacologically inactive [9]. Approximately 90% of the dose of MMF is excreted in the urine as MPAG [10].

The concentration–time profile of MPA following oral administration of MMF often shows two peaks [11, 12]. Maximum concentration (C_{max}) occurs within 1–3 h and

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a second peak is seen at around 6–8 h. This secondary peak is thought to be due to enterohepatic circulation of MPAG back to MPA by glucuronidases from the gastrointestinal flora [13]. It has been suggested that enterohepatic recirculation of MPA is suppressed when it is used in combination with cyclosporin due to the inhibition of MPAG excretion into the bile [14].

MMF displays large between-subject pharmacokinetic variability, with a greater than 10-fold range in MPA area under the concentration–time curve (AUC) following a standard dose [10, 13]. Preliminary pharmacodynamic evidence supports a correlation between MPA $AUC_{0-12\,h}$ and a decreased risk of acute renal rejection [15–18]. In addition, MPA $AUC_{0-12\,h}$ values of 30 mg l^{-1} h and 55 mg l^{-1} h (derived from a fitted curve) have been associated with a 50% and 90% reduction in rejection in renal transplant patients, respectively [10]. In contrast, trough concentrations have been shown to have poor predictive ability [15, 17, 19, 20]. It is unclear which other factors associated with individual subjects (covariates such as age, weight and other characteristics) may influence the pharmacokinetic parameters, which makes dose individualization difficult at present.

The aim of this study was to develop a population pharmacokinetic model for MPA in adult kidney transplant recipients, in order to quantify the average population pharmacokinetic parameters, the variability between and within patients and the influence of covariates on the parameters.

Methods

Patients and data collection

Pharmacokinetic data for MPA and covariate information were available from 22 patients who underwent kidney transplantation at the Princess Alexandra Hospital. MMF was administered orally as 1 g twice per day in all patients. All patients were also receiving cyclosporin and prednisone. Data available for the pharmacokinetic study have been described elsewhere [19, 21]. Briefly, full 12-h plasma concentration–time profiles of MPA were obtained from 10 patients, on three occasions, typically on days 2, 5 and 28 post-transplantation. Fourteen whole blood samples were collected (pre-dose and 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0 and 12.0 h post-dose) on each occasion. In 12 other patients, blood samples were collected on day 2 post-transplant only with blood samples collected at the same times as previously. Whole blood samples were collected into vacuum tubes containing ethylenediaminetetraacetic acid. Plasma was removed after centrifugation (10 min, 1500 ***g***) and stored at −20 °C until analysed. MPA concentrations were determined by a previously validated high-performance liquid chromatography method with ultraviolet detection [22]. It was our premise that the absorption process, rather than the metabolic conversion of MMF to MPA, was the rate-limiting step of appearance of MPA in the body. Patient weight, plasma albumin, serum creatinine and donor status were recorded on each of the study days (2, 5, and 28) for the 10 intensive sampled patients; sex and age were also recorded on occasion one. Age, sex and donor status were also available for some of the additional 12 patients; no other covariates were available for these patients. Demographics are shown in Table 1. A total of 557 concentration–time points were available for population pharmacokinetic analysis.

Table 1 Patient demographics.

Characteristic	*Mean ± SD (range)*	*n**
Age	42 ± 16 (26–65)	11
Sex	*F* = 4, M = 7	11
Weight (kg)	74.2 ± 17.7 (58.3–110)	10
Albumin (g l^{-1})	35 ± 4.4 (29–42)	10
Serum creatinine (mM)	0.24 ± 0.15 (0.12–0.61)	10
Donor status	C = 13, LR = 5, LNR = 1	19

*Number of subjects for whom covariate information was available. C, Cadaver; LR, living related; LNR, living nonrelated.

Exploratory analysis

During exploratory analysis concentration–time profiles for all patients on all occasions were inspected. There was evidence of a lag time in drug absorption, a complex absorption process and enterohepatic recirculation of MPA in some patients on some occasions (examples of concentration–time profiles are shown in Figure 1a,b,c).

Population pharmacokinetic modelling

Data were analysed using the first-order (FO) method in NONMEM (version 5 level 1.1) [23] with the G77 FORTRAN compiler. The first-order conditional estimate with interaction between random effects did not run successfully with this data set and the models we tried and therefore was not used for model building. If minimization did not terminate successfully or the model was unstable (see below), the model was excluded from further model-building steps.

Model selection was based on three criteria. (i) Statistical significance, given by a drop in the NONMEM objective function between successive models based on the χ^2 asymptotic assumption with the number of degrees of freedom equal to the difference in the number of parameters between the full and reduced model pairs (for nested models). The significance level was set at 5%,

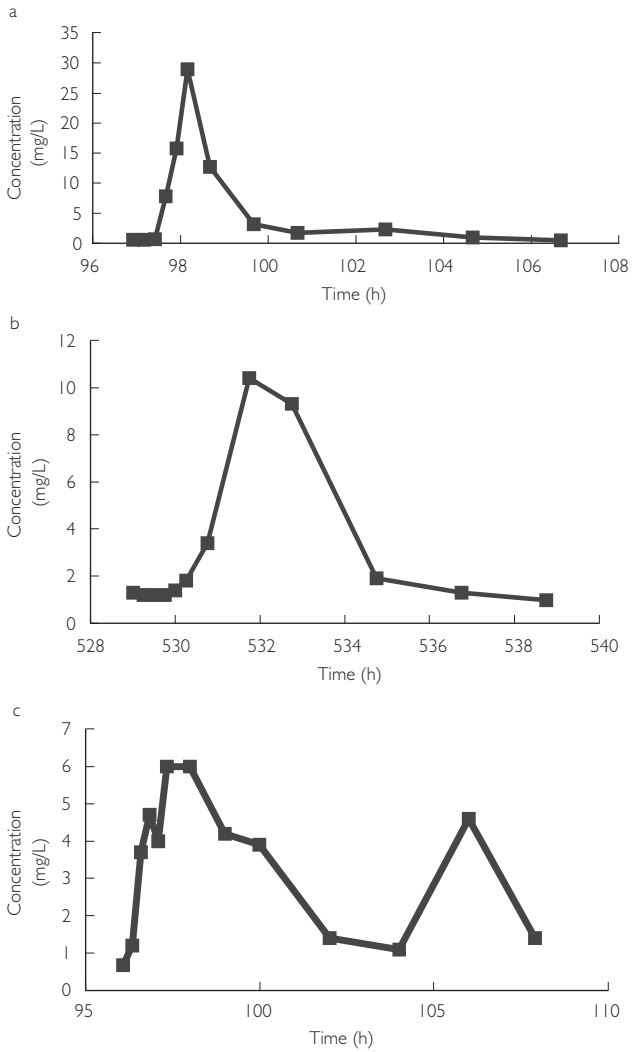

Figure 1 (a) Concentration–time profile of patient 1 occasion 2 demonstrating a lag time in absorption. (b) Concentration–time profile of patient 2 occasion 3 demonstrating a complex absorption process. (c) Concentration–time profile of patient 3 occasion 2 demonstrating enterohepatic recirculation.

which corresponds to an approximate drop in the objective function of 3.8 units per one parameter difference in successive models. Since the FO method in NONMEM is known to be anticonservative with respect to this procedure [24], then a larger drop in the objective function was considered desirable to indicate potential significance. We, arbitrarily, chose a 5-unit decrease but are aware that the critical value for the χ^2 statistic may be much greater. (ii). Plausible results. Pharmacokinetic parameter estimates were required to be legal and physiologically plausible (e.g. clearance could not be negative and volume of distribution could not be <3 l). In addition, the between- and within-subject variance values expressed as a coefficient of variation were required to fall between 1 and 500% (values outside this range are often indicative of over-parameterization rather than a true representation of the variability). (iii) Model stability was assessed by two methods: first, changing the number of significant digits, and second, changing the initial estimates of the parameters. In both circumstances these tests should not significantly affect the final parameter estimates if the model is stable.

For the covariate analysis, two additional criteria were also considered: (i) clinical significance. The influence of the variability in the covariate had to have a clinically significant effect on the value of the parameter (i.e. >20%); and (ii) the relationship was required to be biologically plausible. Twenty percent was chosen arbitrarily as the cut-off for clinical significance.

Model development

Structural and statistical models All compartmental models were parameterized in terms of clearance and volume terms with rate constants only used to describe the absorption process. Clearance and volume terms were modelled as apparent oral value (e.g. CL/F), but for simplicity the oral availability term (F) is not shown in future notation.

Enterohepatic recirculation As it is widely accepted that MPAG undergoes enterohepatic recirculation back to MPA, and features of enterohepatic recirculation in the concentration–time profile for some patients in the exploratory analysis were seen, a model describing enterohepatic recirculation was considered. This model allowed estimation of the fraction of MPA excreted into the bile (F_{EH}) and the time of gallbladder emptying (T_{EH}). Several assumptions were made to ensure the model was structurally identifiable: (i) only one recirculation process occurred per dose interval; (ii) elimination of the drug into the bile from the intestine was assumed to be at the same rate as other elimination processes; (iii) the fraction of drug in the gallbladder remained constant until the gallbladder was emptied; and (iv) the rate of emptying of drug back into the intestine was assumed to occur at the same rate as absorption from the intestine. This last assumption implies that the rate-limiting step for the input of MPA into the systemic circulation (via both oral administration of MMF and the enterohepatic recirculation of MPAG) is due to absorption rather than conversion to MPA. In addition, this assumes that the absorption of MMF and MPA would be similar. Although this was not strictly necessary, it was seen in the final model that these parameters are difficult to identify independently.

Statistical model for the data Various statistical models to describe the data were considered:

$$y_{ij} = f(\theta_i, x_{ij}) + \varepsilon_{ij} \quad (1)$$

$$y_{ij} = f(\theta_i, x_{ij}) \times e^{\varepsilon_{ij}} \quad (2)$$

$$y_{ij} = f(\theta_i, x_{ij}) \times e^{\varepsilon 1_{ij}} + \varepsilon_{2ij} \quad (3)$$

Where y_{ij} is the j^{th} observed concentration for the i^{th} individual, $f(\theta_i, x_{ij})$ is the model-predicted concentration and ε_{ij} is the difference of the j^{th} observed concentration for the i^{th} individual from the expected concentration and it is assumed that ε_{ij} are independent and identically distributed of the form $\varepsilon \sim N(0, \sigma^2)$.

Between-subject variability (BSV) Between-subject pharmacokinetic variability was assumed to be log normally distributed and was modelled.

$$\theta_i = \theta \times e^{\eta i} \quad (4)$$

Where θ_i is the i^{th} subjects value of the parameter, θ is the typical value in the population, and η_i are independent and identically distributed of the form $\eta_i \sim N_{pB}(0,\Omega_B)$, and Ω_B is the variance–covariance matrix of between-subject effects (p_B denotes the number of between-subject random effects).

Within-subject variability (WSV) Within subject variability is the variability of a parameter within a subject during treatment and comprises between-occasion variability (BOV) and within-occasion variability. However, in general, within-occasion variability can not be estimated, hence only BOV is considered. BOV was assumed to be log normally distributed and modelled over successive study days.

$$\theta_{ik} = \theta \times e^{\eta i + \eta ik} \quad (5)$$

Where θ_{ik} is the i^{th} subjects value of the parameter on the k^{th} occasion, θ is the typical value in the population, η_{ik} are independent and identically distributed of the form $\eta_{ik} \sim N_{pW}(0,\Omega_W)$, and Ω_W is the variance–covariance matrix of within-subject effects (p_W denotes the number of within–subject random effects).

Covariate analysis

A four-step approach was used to identify the influence of covariates, since for some patients most of the covariate information was missing completely. It should be noted that modelling missing covariate values in our circumstance, i.e. (i) when covariate data were missing it was missing for almost all covariates, and (ii) over half the study patients had missing covariates, is likely to yield relationships that may be imprecise. We performed this analysis to provide an insight as to which covariates should be considered in future population analyses from a design perspective and not for the purposes of predictions for new patients.

1. An exploratory analysis was initially conducted in the 10 patients (reduced data set) who had complete covariate data available. Empirical Bayes (POSTHOC) parameter estimates from the base model from the reduced data set were plotted against covariate values to assess relationships. Covariates that provided visual relationships were examined further in step 2.

2. The relationship between covariates and pharmacokinetic parameters was statistically assessed using NONMEM in the 10 patients (reduced data set). The most important covariate regression relationships were examined further in step 3. The process of joint function modelling presented in step 3 requires that the structural form of the covariate regression relationship be known *a priori*. This is essential so that parameter values and missing covariates can be estimated simultaneously based on the structure of the defined relationship. In this example, the structural form of the regression relationship is only able to be determined for the reduced data set.

3. To compensate for missing covariates in the other 12 patients, a joint function modelling approach was used in NONMEM. This approach estimated the missing covariate values for each of the 12 patients simultaneously with estimates of their pharmacokinetic parameter values. Due to a large number of missing covariates, only the single most influential covariate was considered. During joint function modelling, population values, BSV and BOV of the covariate were fixed and only the individuals' covariate values estimated. The best model, defined by a statistically significant change in objective function, was retained as well as the estimates of the (missing) covariates. Since the objective function of NONMEM in this part of the analysis contains information on both the concentration observations and missing covariates, comparison with the base model from the full data set to assess for the statistical significance of the covariate relationship is not possible.

4. The imputed covariate values (from step 3) were entered into a copy of the original data file, thereby creating a new data set identical to the original in every way except for containing a complete set of covariate values for all patients. Covariate values that were already known were not re-estimated. The number of evaluations was set to zero (MAXEVAL = 0), and the objective function between full and base models compared.

Results

Structural model – basic model building

Using a one-compartment model, the data were best described with a mixed residual unexplained variance

(RUV) model. BSV for distributional clearance (Q) was very large (i.e. >500), therefore only estimation of the mean population parameter value was considered. A two-compartment model with a full variance covariance matrix was approximately 82 units lower in the objective function when compared with the one-compartment model. The objective function for a three-compartment model estimating only the diagonal of the variance covariance matrix did not improve the fit further. The data were not able to statistically support a model accounting for enterohepatic recirculation. Models accounting for the complex absorption process, e.g. time-dependent absorption, E_{max} and Weibull and a dual sequential first-order absorption process, did not improve the fit statistically. Addition of lag time, however, provided a significant improvement.

Base model with BOV

A model accounting for BOV with a full variance covariance matrix displayed a significantly lower objective function, although the minimization terminated. The best model accounting for occasion to occasion variability incorporated only the diagonal elements of the variance covariance matrix. Further attempts to estimate various permutations of the variance covariance matrix did not improve the fit further. The decrease in objective function from the previous to the final base model was 146 units (dof = 5), and the smallest drop in objective function between any successive models was 15 units (dof = 1).

Model diagnostics

Diagnostic plots of were assessed visually (Figure 2a–e). Most of the weighted residuals were within ±2 SD of the mean (Figure 2a–c). Plots of population observed concentrations and predicted concentration *vs.* time suggested the model described the data well (Figure 2e). However, this model would not be expected to describe the complex input process depicted in Figure 1b.

From diagnostic plots of the empirical Bayes (POSTHOC) parameters estimates *vs.* occasion, it appeared *CL* of MPA increased with time. This was statistically significant ($P < 0.05$) when using a paired *t*-test of the empirical Bayes estimates of *CL* on occasion 3 (day 28) with occasion 1 (day 2). The apparent change in clearance could be a function of an increase in clearance over time or saturable protein binding resulting in greater MPA free fraction on day 28 and a decrease in total concentration. Although the latter approach is a plausible explanation for the change in total concentration, and hence apparent change in clearance, there are no pharmacokinetic data supporting such a phenomenon. Available *in vitro* evidence suggests that the dissociation constant (K_D) of MPA for albumin is approximately 100 times greater than the concentrations seen in this study [25]. This model was not considered further.

Time-dependent clearance

Two empirical models for time-dependent *CL* within the two-compartment structural model were considered.

$$\text{Clearance} = CL_{BL} + (CL_{MAX} \times t)/(t_{50} + t) \quad (6)$$

$$\text{Clearance} = CL_{BL} + CL_{MAX}(1 - e^{-KCL \times t}) \quad (7)$$

Where CL_{BL} is the base rate clearance, CL_{MAX} is the maximum rate of clearance, t is time, K_{CL} is the association rate constant and t_{50} the time to achieve 50% of maximum clearance.

Both models for time-dependent clearance resulted in a reduction in the objective function of approximately 20 units ($P < 0.05$). However, both proved unstable, with their objective function and parameter values changing depending on the initial starting estimates.

Confidence interval of parameter estimates for the final base model

The final base model was a two-compartment model with a lag time in the absorption phase and BOV. Results from the best final model are presented in Table 2. For parameters that had large standard errors or for variance estimates (BSV or BOV) that were larger than 100%, we computed the 95% profile likelihood confidence interval, shown in Table 3. The profile likelihood method suggested that the BSV of *CL and* the residual variance were estimated well. The BOV for *KA*, *V3 and* lag time were larger, indicating that there was less information about these parameters in the design. Not unexpectedly, the asymptotic estimates of the confidence interval often included illegal parameter values.

Covariate analysis

Step 1 Covariate testing was initially performed in a reduced data set of 10 patients who had all covariate values recorded. When the empirical Bayes parameter estimates were plotted against covariate values it appeared that *CL and V2* were weakly influenced by patient weight, but not by other covariates (Figure 3). Relationships of *CL* with patient weight, *V2* with patient weight and *CL and V2* with patient weight were investigated further.

Step 2 Two weight-based covariate relationships (equations 10 and 11) were developed to explore the effect of weight on *CL and V2*.

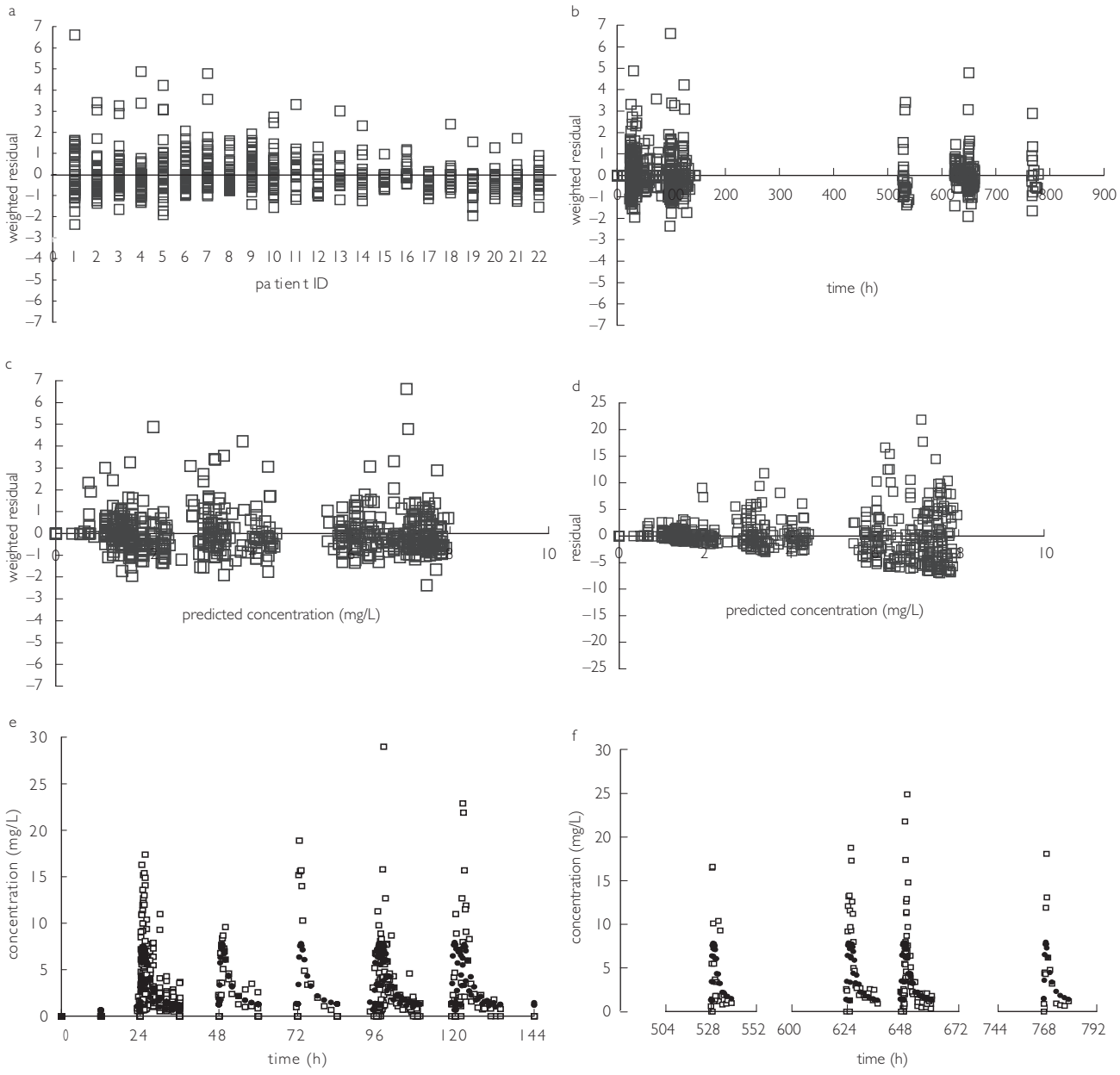

Figure 2 Model diagnostics of the final base model. (a) Scatter plot of weighted residual *vs.* patient ID. (b) Scatter plot of weighted residual *vs.* time. (c) Scatter plot of weighted residual *vs.* predicted mycophenolic acid (MPA) concentration (mg l^{-1}). (d) Scatter plot of residual *vs.* predicted MPA concentration (mg l^{-1}), and (e) a scatter plot of predicted (●) and observed (□) concentration *vs.* time.

$$\theta_1 = \theta^1 \times (Weight/70) + \theta^2 \quad (8)$$

$$\theta_1 = \theta^1 \times (Weight/70)^{0.75} + \theta^2 \quad (9)$$

Where θ_i is the i^{th} patient's value of θ. In both cases, a reduced model was generated by setting θ^1 to zero. The estimate of the parameters at this part was used as initial estimates for the joint function modelling step (step 3).

Step 3: joint function modelling Equation 8 provided the best relationship from step 2 and was used to assess the covariate relationship in the joint function modelling process. *CL vs.* weight achieved the lowest objective function. Since there were two parameters that appeared to be influenced by weight then there were three possible relationships, *CL* on weight or *V2* on weight and both *CL* and *V2* on weight. When performing the joint function modelling, values of weight are 'imputed' based on their relationship with the parameter simultaneous with the parameter being estimated. In essence, the model predicts weight and the parameter values from the data simultaneously and hence a joint function is modelled. When the POSTHOC feature of NONMEM is used the

Table 2 Summary of results of the final base model.

Fixed effects parameters					
Clearance	*CL* (l h^{-1})		27.1 (1.42)		
Central volume of distribution	*V2* (l)		97.7 (12.6)		
Absorption rate constant	*Ka* (h^{-1})		2.27 (0.184)		
Distributional clearance	*Q* (l h^{-1})		25.7 (3.45)		
Peripheral volume of distribution	*V3* (l)		206 (55.7)		
Lag time	lag (h)		0.145 (0.0201)		
Between-subject variability (Ω_B)					
	ω_{CL}	ω_{V2}	ω_{Ka}	ω_{V3}	ω_{lag}
ω_{CL}	0.0396 (0.0245)				
ω_{V2}	–	0.309 (0.180)			
ω_{Ka}	–	–	0		
ω_{V3}	–	–	–	2.27 (2.22)	
ω_{lag}	–	–	–	–	0
Between-occasion variability (Ω_W)					
	ω_{CL}	ω_{V2}	ω_{Ka}	ω_{V3}	ω_{lag}
ω_{CL}	0.0175 (0.013)				
ω_{V2}	–	0.912 (0.267)			
ω_{Ka}	–	–	3.4 (0.701)		
ω_{V3}	–	–	–	0.26 (0.234)	
ω_{lag}	–	–	–	–	0.106 (0.364)
Residual unexplained variance					
	σ_1^2 (exp)		σ_2^2 (add)		
σ_1^2	0.123 (0.0759)				
σ_2^2	–		0.57 (0.503)		

Standard errors shown in parentheses.

Table 3 Confidence intervals for selected parameters from final base model.

	Between–subject variance		*Within-subject variance*		*RUV*	
	CL	*V3*	*Ka*	*V3*	*Lag*	σ_2^2
Mean value	0.0396	2.27	3.40	0.260	0.106	0.570
Asympotic standard error	0.0245	2.22	0.701	0.234	0.364	0.503
Asymptotic 95% CI	– 0.008 to 0.088	– 2.08 to 6.62	2.03–4.77	– 0.199 to 0.72	– 0.607 to 0.82	– 0.416 to 1.56
Profile likelihood 95% CI	0.013–0.106	0.430–6.43	2.07–5.74	> 0 to 1.52⋆	> 0 to 0.573⋆	0.410–0.776

95% CI = 95% confidence interval. ⋆> 0 depicts a value that is just greater than zero.

values of weight for those patients in whom the weight was missing are provided. These values will depend on the covariate relationships being tested. Since model 8 was preferred then there are three possible covariate relationships between weight and *CL* and *V2*, hence three sets of missing covariates were imputed. Each set was slightly different in accordance with the differing regression relationships.

Step 4: full data set with imputed missing covariates Three new data sets with complete weight values were created from step 3. Each of the full data sets was then used to assess the relationship between *CL* or *V2* and both *CL* and *V2* with patient weight. The lowest objective function was achieved by *CL* and *V2 vs.* patient weight. None of the relationships with weight reached statistical significance. The maximum difference in the objective functions was 3 points. The slope coefficients (θ^1) did suggest, however, that a potentially clinically significant relationship might have existed, although given the amount of missing covariate information this should be viewed with caution.

Discussion

This is the first reported population pharmacokinetic analysis of mycophenolic acid. Currently, most pharmacokinetic studies of MPA have used noncompartmental analysis with subjects receiving multiple doses of MMF and the analysis completed independently on each occasion. In some of these studies the MPA $AUC_{0-12\,h}$ were found to correlate with clinical outcomes [20, 26].

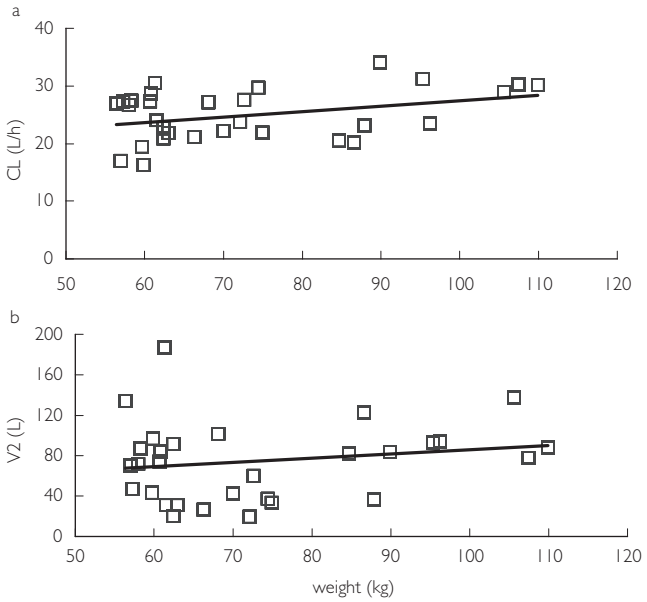

Figure 3 Scatter plot of empirical Bayes parameter estimates *vs.* covariates for (a) *CL vs.* weight and (b) *V2 vs.* weight.

Population values of *CL*, *V2*, *Ka*, *Q*, *V3* and lag time in absorption have been quantified in this present study for 22 renal transplant recipients (Table 2). Additionally, the between-subject variability and between-occasion variability of these pharmacokinetics parameters have been characterized. It is noted that *V2* and *Ka* have particularly large BOV and *V3* has large BSV. NONMEM was not able to provide an estimate of the BSV for lag time, but potentially significant BOV was seen.

MPA demonstrates complex pharmacokinetics. There appears to be evidence of a variety of processes, but the data from this study often could not support many of the more complex disposition models. This was demonstrated by both time-dependent clearance models that led to a statistically significant decrease in objective function when compared with a two-compartment model. However, neither of these models were stable. In addition, it is possible that time-dependent changes in *CL* may be confounded with and potentially inflate the estimation of BOV, although the extent of the influence of this in this data set is unclear. The enterohepatic recirculation model was successfully implemented but the differences did not reach statistical significance. In addition to the apparent low power of the design to support these features of the pharmacokinetic profile, the study was unable to characterize the effects of comedications. Current literature suggests co-administration of cyclosporin and MMF may inhibit the enterohepatic recirculation of MPA [27–29], and there are also data indicating an effect of corticosteroids on the pharmacokinetics of MPA [30].

This study did not identify a significant covariate relationship. It is likely that this is due to the large amount of missing covariates, where only 10 out of 22 patients had serum creatinine, plasma albumin and weight recorded. Interestingly, our results potentially contrast with other studies where weight has not been described to influence the pharmacokinetics of MPA [13]. However, from the exploratory analysis, the effect of weight was found to be the most significant covariate (albeit weak). The most significant covariate relationships were (i) *V2 vs.* weight in the reduced data set of 10 patients, (ii) *CL vs.* weight in the joint function modelling, and (iii) V2 *vs.* weight in the full data set of 22 patients. None of these results reached statistical significance, and dose adjustment for weight should not be considered on the basis of these analyses. We do, however, recommend that weight be considered as a potential covariate when designing future pharmacokinetic studies.

Several review articles have discussed a number of limited sampling strategies for the estimation of MPA $AUC_{0-12\,h}$ due to the impracticality of a full blood sample collection strategy [10, 19, 31]. From this study, large between-subject and between-occasion variability of the pharmacokinetics of MPA has been described. Given the evidence of variable lag time in the absorption of MPA and the potential for time-dependent clearance, the usefulness of a limited sampling strategy approach for MPA is questionable. For example, the population mean lag-time value was 10 min and the between-occasion variability approximately 33%. The delay in absorption could range from zero to up to 35 min. Many current limited sampling strategies have one or more early sampling times in the first hour, often at 0.25 and 0.75 h [19], which, given the variable lag time for absorption for an individual from one day to another, may significantly reduce the predictive power of these methods. Interestingly, Willis [19] showed that the limited sampling strategy whose first sampling time was at 1 h (with three additional times) provided significantly better estimates of the AUC when tested prospectively than did the other strategies, four of which had early sampling times within the first hour.

Future studies should attempt to investigate further the nonlinearity in the disposition of MPA. Models to describe the change in clearance with time (which was not successfully described in this study) using time-dependent clearance or more mechanistic models that account for changes in the underlying metabolic processes should be considered. Covariate relationships remain to be confirmed. It is possible that weight will prove to be influential in subsequent analyses, as may concomitant medications and their doses, and therefore these should be considered in any future pharmacokinetic study design. Population pharmacokinetic studies with a larger number of patients and full covariate information, and perhaps measurement of free drug concentrations,

are needed to gain a clearer picture of the influence of covariates on the pharmacokinetic profile of mycophenolate acid.

In conclusion, this study has increased the current understanding of MPA pharmacokinetics. Population pharmacokinetic parameters were quantified. The complexity in determining the pharmacokinetics of MPA is currently underestimated and more complex pharmacokinetic models may prove useful in the future. The large between-subject and -occasion variability and complexity in the input and disposition of MPA raise questions about the value of the use of therapeutic monitoring and limited sampling strategies as currently practised.

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