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PHARMACOKINETICS AND DISPOSITION

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Population pharmacokinetics of mycophenolic acid during the first week after renal transplantation

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Abstract *Objective*: To investigate the population pharmacokinetics of mycophenolic acid (MPA) in adult kidney transplant recipients during the crucial first week after transplantation.
Methods: Data were collected from 117 patients. MPA plasma concentrations were determined at $t = 0$, 1, 2, 3 and 4 h after mycophenolate mofetil dosing on days 3, 5 and 7. Population analysis was performed using NONMEM. Covariates screened were sex, age, body weight, serum creatinine, creatinine clearance, serum albumin, days of therapy, diabetes mellitus, organ source (live or cadaveric) and co-therapy (tacrolimus or cyclosporine). Final model validity was evaluated using 200 bootstrapped samples from the original data. Bias and precision were determined through comparison of observed and predicted concentrations.
Results: Individual concentration–time profiles showed evidence of an absorption lag time and enterohepatic recirculation of MPA in some patients on some occasions. The best base model had bi-exponential elimination with a typical population (SE%) apparent clearance (CL/F) of 29 l/h (5%) and apparent volume of the central compartment of 65 l (7%). CL/F decreased significantly with increasing serum albumin (1.42 l/h reduction in total plasma CL/F with each 1 g/l increase in albumin) and was 27% greater in patients receiving cyclosporine than in those receiving tacrolimus. Evaluation of the final model showed close agreement between pairs of bootstrapped and final model parameter estimates (all differences $< 7\%$). Predictions were non-biased (0.11 mg/l) but imprecise (2.8 mg/l).
Conclusion: Population pharmacokinetic parameters for MPA were determined. These can be used to achieve specific target MPA concentrations or areas under the concentration–time curve.

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Introduction

Mycophenolate mofetil (MMF) is widely used in immunosuppressive regimens with corticosteroids and calcineurin inhibitors to reduce the incidence of acute rejection. Following oral administration, MMF undergoes rapid absorption and hydrolysis to mycophenolic acid (MPA), the active immunosuppressive metabolite [1]. Unlike other immunosuppressants, MMF has conventionally been administered as a fixed dose without routine monitoring of MPA.

Despite this fixed dosage strategy, MMF displays large between-subject pharmacokinetic variability, with a greater than tenfold range in MPA area under the plasma concentration–time curve (AUC) in individuals receiving the same standard dose [2–4]. Several studies have shown a relationship between MPA AUC and biopsy-proven rejection [5–8]. A relationship between MPA AUC and toxicity has also been demonstrated [3, 4], but is not as clear. There is a growing consensus favouring therapeutic monitoring of MPA, adjusting doses to specific AUC targets, especially in the initial post-transplant phase [3, 4, 7, 9, 10].

Measuring 12-h MPA AUCs is impractical in the clinical setting. Limited blood sampling (at 0, 1, 3 and 6 h) correlates with $AUC_{(0\text{-}12)}$ closely ($r^2 = 0.954$) and accurately [11]. However, even this strategy is not feasible in all situations, especially after hospital discharge. It may be preferable to use one or perhaps two MPA concentrations, collected at known but non-rigid times, in a Bayesian estimation method, to individualise dosage to specific target AUC for each transplant recipient [12].

The Bayesian estimation approach requires initial population pharmacokinetic information on MPA. Population analysis provides population pharmacokinetic parameter estimates, estimates of within- and between-subject variability in these parameters and allows patient characteristics explaining between-subject variability (covariates) to be quantified [12].

To date, two population pharmacokinetic analyses of MPA have been published [13, 14]. Shum et al. [13], developed a model in 22 adult kidney recipients using MPA concentrations typically taken on days 2, 5 and 28 post-transplant. The best base model was a two-compartment model with a lag time (mean apparent clearance of 27 l/h and apparent volume of the central compartment of 98 l). Le Guellec et al. [14] developed a model in 60 stable adult kidney recipients at least 6 months post-surgery. A two-compartment model with zero-order absorption best fitted the data (mean apparent clearance of 0.246 l/h/kg and apparent volume of the central compartment of 36 l). Apparent clearance positively correlated with body weight; however, its inclusion in the model caused only a minor reduction in the estimated between-subject variability.

A number of factors have been reported, or suggested, to alter the pharmacokinetics of MPA, including concomitant immunosuppressant administration (tacrolimus or cyclosporine), food intake, analytical technique for MPA measurement, age (child versus adult) and time after transplant [9]. It is likely that MPA pharmacokinetics will be especially variable early post-transplant when delayed graft function, hypoalbuminaemia, hyperbilirubinaemia, altered gastrointestinal motility, widely varying drug absorption and large immunosuppressant dosage is common. To date, no population pharmacokinetic study has focussed on this critical early post-transplant phase.

One factor likely to complicate population modelling during this period is changing MPA free fraction. MPA is extensively bound to albumin, with protein binding reported to be in the order of 97–99% in patients with normal renal and liver function [1, 15, 16]. MPA-glucuronide (MPAG), the main metabolite of MPA, is approximately 82% bound in stable patients. Significant renal dysfunction, liver disease and hypoalbuminaemia can alter MPA and MPAG binding, changing the fraction of free drug. However, changes in total concentration of MPA (e.g. due to increased drug dosage) may not be associated with parallel changes in free concentration [9, 15–17]. This is important, as the concentration that is determined in clinical practice is the total MPA plasma concentration, and this may not truly reflect changes in the free concentration.

Hypoalbuminaemia is probably the most important factor influencing MPA free fraction. In vitro, MPA free fraction increased approximately 41-fold when human serum albumin decreased from its normal physiological concentration of 41.4 g/l to a very low 0.07 g/l [15]. Increase in free fraction was 2.2-fold when serum albumin decreased to 20.7 g/l [15]. Increased free-fraction MPA has also been associated with decreased serum albumin in vivo [17, 18]. In 42 adult kidney transplant recipients, Atcheson et al. [18] identified a cut-off value for serum albumin of 31 g/l, below which free-fraction MPA was significantly elevated [defined as ≥3%; 20% above the reported upper normal limit (1–2.5%)].

The purpose of this study was to investigate the population pharmacokinetics of MPA during the first week after kidney transplantation, to quantify the population pharmacokinetic parameters, between-subject and between-occasion variability, and to identify which factors (covariates) have significant influence on pharmacokinetic variability during this period.

Subjects and methods

Data were collected from 117 renal transplant recipients at the Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia, Canada, who had up to three MPA AUCs measured during their first week of MMF therapy. Recipients had blood samples collected at $t=0$ (trough, pre-dose), 1, 2, 3 and 4 h after dosing (limited or abbreviated AUCs rather than full AUCs). These samples were collected as part of routine clinical care on days 3, 5 and 7 post-transplant.

Patients received oral MMF (Cellcept, 500-mg tablets) as part of a 'triple' immunosuppressive regimen, which also included corticosteroids and cyclosporine or tacrolimus. Therapy was initiated at a dose of 2 g daily, administered in two divided doses, 12-hourly.

MPA plasma concentrations were analysed in the TDM laboratory, Queen Elizabeth II Health Sciences Centre, Halifax, Nova Scotia, Canada. The method of Svensson [19] was followed with slight modification. Specimens were collected in glass collection tubes containing ethylene diamine tetraacetic acid as an anticoagulant. Proteins were removed by precipitation using acetonitrile containing the internal standard (carboxy butoxy ether of mycophenolate—CBEM). After centrifugation, an aliquot of the supernatant was injected onto a high-performance liquid chromatography RP-18 column with ultraviolet detection at 215 nm. The limit of quantification was 0.4 mg/l and there was a day-to-day imprecision (CV) of 2.8% at 2.0 mg/l ($n=20$) and 1.8% CV at 7.5 mg/l ($n=20$). Performance of the MPA assay was monitored externally by enrolment in the International Mycophenolate Proficiency Testing Programme, St. George's Hospital Medical School, London, UK (Dr. David Holt, http://www.bioanalytics.co.uk).

Human Research Ethics Committee approval was obtained (UQ approval number:#2002000477) and data were analysed using the population pharmacokinetic package NONMEM (version V). FORTRAN compilation was achieved with the G77 compiler. Analyses were performed using the First Order Conditional Estimation (FOCE) algorithm with interaction.

Model selection was based on goodness-of-fit in addition to the three criteria of statistical significance, plausibility and stability. Models were compared statistically using a likelihood ratio test on the differences in the objective function (OF) value. Statistical significance was set at $P < 0.01$. Plots of residuals and weighted residuals, standard errors of parameter estimates and changes in estimates of between-subject and residual variability were also examined.

Pharmacokinetic parameter estimates were required to be physiologically plausible. A model had to remain stable when significant digits and initial parameter estimates were altered. Clinical significance and biological plausibility of covariate addition was also considered. A 20% change was chosen as the cut-off for clinical significance.

Preliminary analyses focussed on the structural and variance model. Single- and bi-exponential elimination models with first-order absorption were tried. All compartmental models were parameterised 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 values (e.g. CL/F, V/F). Use of a lag time for drug absorption and fixing the absorption rate constant k_a was also examined. Between-subject and between-occasion variability were assumed to have a log-normal structure. Residual random error was modelled using additive, exponential and combined error structures.

As it is widely accepted that MPAG undergoes enterohepatic circulation back to MPA, a model describing this process was also tested [13]. This model allowed estimation of the fraction of MPA excreted into the bile and the time of gallbladder emptying.

Covariates screened for influence on the pharmacokinetic parameters were sex, age, body weight, serum creatinine (day 7), creatinine clearance (day 7), serum albumin [day 3 and day 5, within-subject variability, mean ($\pm$SD) percentage difference between the two measured albumin concentrations was $4 \pm 14\%$ ($n = 68$)], days of therapy, whether the patient was diagnosed as having diabetes mellitus, organ source (live or cadaveric) and co-therapy [tacrolimus or cyclosporine (Neoral)]. Creatinine clearance was calculated from serum creatinine values using the standard Cockcroft and Gault equation. Information on patient height was not available so ideal body weight could not be applied in estimates of patient size and creatinine clearance.

During preliminary analysis, covariate correlations and relationships were investigated statistically. Covariate influences on pharmacokinetic parameters were also examined by plotting empirical Bayes estimates of individual parameters against covariates. Covariates identified as potentially influencing pharmacokinetic parameters were then tested formally in NONMEM.

Significant covariates were cumulatively added to the model in a stepwise, descending order of potential influence on model parameters in accordance to their contribution to the reduction in OF in the initial analysis, until there was no further significant reduction. Finally, a stepwise backwards elimination step was performed in which the influence of each covariate was removed from the model by setting the appropriate coefficient to zero and re-estimating parameters in the model. This procedure was performed in descending order of the contribution to the change in OF by each covariate, as determined in the initial analysis. Statistical significance for forwards addition and backwards elimination was set at $P < 0.01$ (a reduction in OF of ≥ 6.63, for one degree of freedom).

Theoretically, changes in MPA free fraction due to changes in albumin should influence all pharmacokinetic parameters estimated from total concentrations except rate constants involved with drug absorption. As an alternative to modelling serum albumin as a covariate for individual pharmacokinetic parameters (see above), the effect of its incorporation into the best base model with an albumin-dependent scaling of concentration was also examined using the following equation:

$$\begin{aligned} Y1 &= Y2 \times ALB \times D_i \\ D_i &= \theta_1 \times e^{\eta i^1} \\ Y &= (Y1 + Y2) \times e^{\varepsilon ij} \end{aligned}$$

where Y1 represents plasma-bound MPA concentration; Y2 free MPA concentration; (Y1 + Y2) total MPA concentration; ALB serum albumin concentration in g/l; D_i a multiplying factor in the relationship between serum albumin and plasma concentration that varies by $e^{\eta i^1}$ between individuals, and ϵij the difference in the jth observed concentration for the ith individual from the expected concentration, which is assumed to be randomly distributed with a mean of zero and variance of σ^2.

The final population model was assessed using a bootstrap method [20]. In brief, this involves repeated random sampling, with replacement, of the original data set to produce another data set of the same size but with a different combination of subjects. As the number of bootstrap samples approaches infinity, the sample standard deviations of the parameters approach the "true" (but unknown) standard deviations.

In this study, bootstrapping was performed using the software program Wings for NONMEM (developed by N. Holford, University of Auckland. http://wfn.sourceforge.net/). Mean parameter estimates obtained from 200 bootstrap runs were compared with population mean values.

Bias (expressed as the mean predicted error of observed and model predicted concentrations) and precision (root mean square prediction error) associated with observed and predicted concentrations from the final population model were also calculated [21]. These values were compared with mean bias and precision associated with observed and predicted concentrations from the 200 bootstrapped analyses.

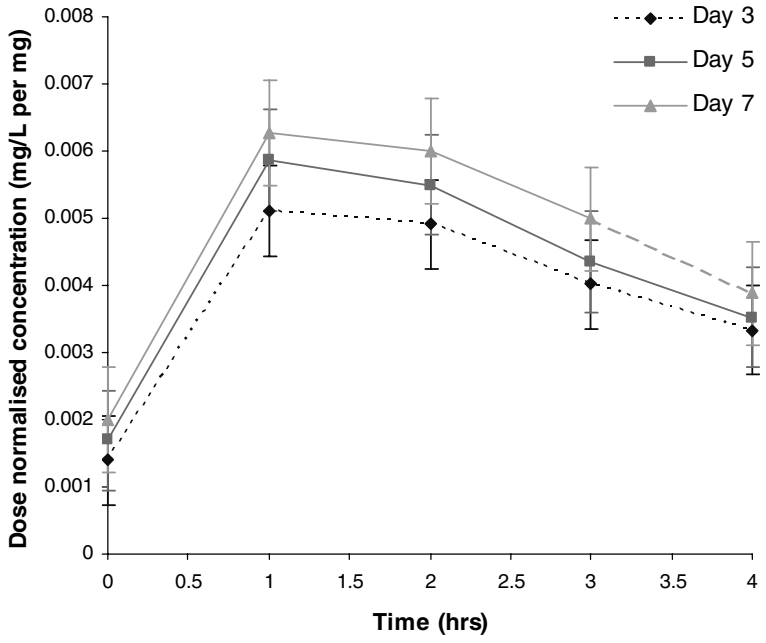

Fig. 1 Mean dose normalised mycophenolic acid concentration (±SE) versus time post-dose on days 3, 5 and 7 after transplantation ($n = 1{,}376$)

Results

Demographic data are presented in Table 1. Of the subjects, 36% received a kidney transplant due to glomerulonephritis, 20% polycystic kidney disease, 12% diabetes mellitus, 8% interstitial nephritis, 7% a congenital disease, 8% hypertension, 2% drug toxicity, 3% for other reasons and for 5% the reason was unknown. A total of 1,376 concentration–time measurements were collected. The number of MPA concentrations per patient ranged from 2 to 15 samples, with a mean (±SD) of 11.8 ± 3.5; concentrations ranged from 0.4 mg/l to 24.2 mg/l, with a mean of 4.0 ± 3.2 mg/l. Samples were drawn from 0 h to 4 h post-dose (Fig. 1).

Preliminary analysis

Information on patient sex, organ source (live or cadaveric), whether the patient was a diabetic and immunosuppressant co-therapy were available from all 117 patients. Weight and age were missing for two patients, serum creatinine for one and albumin for 13. These few missing covariate values were imputed via multiple regression analysis using information gained during preliminary analysis of covariate correlations and relationships.

As an initial step before modelling, the raw data was first examined. Limited concentration–time profiles were plotted for each patient and inspected to see what information they might provide. There was evidence of a lag time in drug absorption, complex absorption processes and enterohepatic circulation of MPA in some patients on some occasions (examples are shown in Fig. 2).

Serum creatinine and patient age showed minimal correlation with patient weight ($r^2 = 0.0795$) and ($r^2 = 0.0503$), respectively. Diabetics ($n = 98$) were 15 kg heavier than non-diabetics ($n = 19$) ($P = 0.0017$), males ($n = 72$) were 11 kg heavier than females ($n = 45$) ($P = 0.0025$), and recipients of an organ from a cadaveric donor ($n = 90$) had a 23% lower creatinine clearance ($P = 0.00295$) and were 8 years older ($P = 0.00823$) than recipients of an organ from a live donor ($n = 27$). Males ($n = 64$) had a 7% higher serum albumin than females ($n = 40$) ($P = 0.0246$).

Population modelling

A bi-exponential elimination model with first-order absorption and proportional residual error was considered superior to a single-exponential elimination model

Table 1 Characteristics of study patients [number, or median (range)]

Demographic data	
Number	72 males, 45 females
Age (years)	50 (19–72)
Weight (kg)	81 (37–134)
Transplant type	90 Cadaveric, 27 living-related
Transplant number	100 first, 16 second, 1 fourth
Serum creatinine (mmol/l)	0.15 (0.06–1.03)
Creatinine clearance (ml/min)	55 (7.6–179)
Albumin (g/l)	26 (6–44)
Total samples	1,376
Dose (g/12 h)	1 (0.5–1)
Concentrations (mg/l)	3.2 (0.4–24.2)
Samples per patient	11 (2–15)
Follow-up period (days)	7 (3–7)

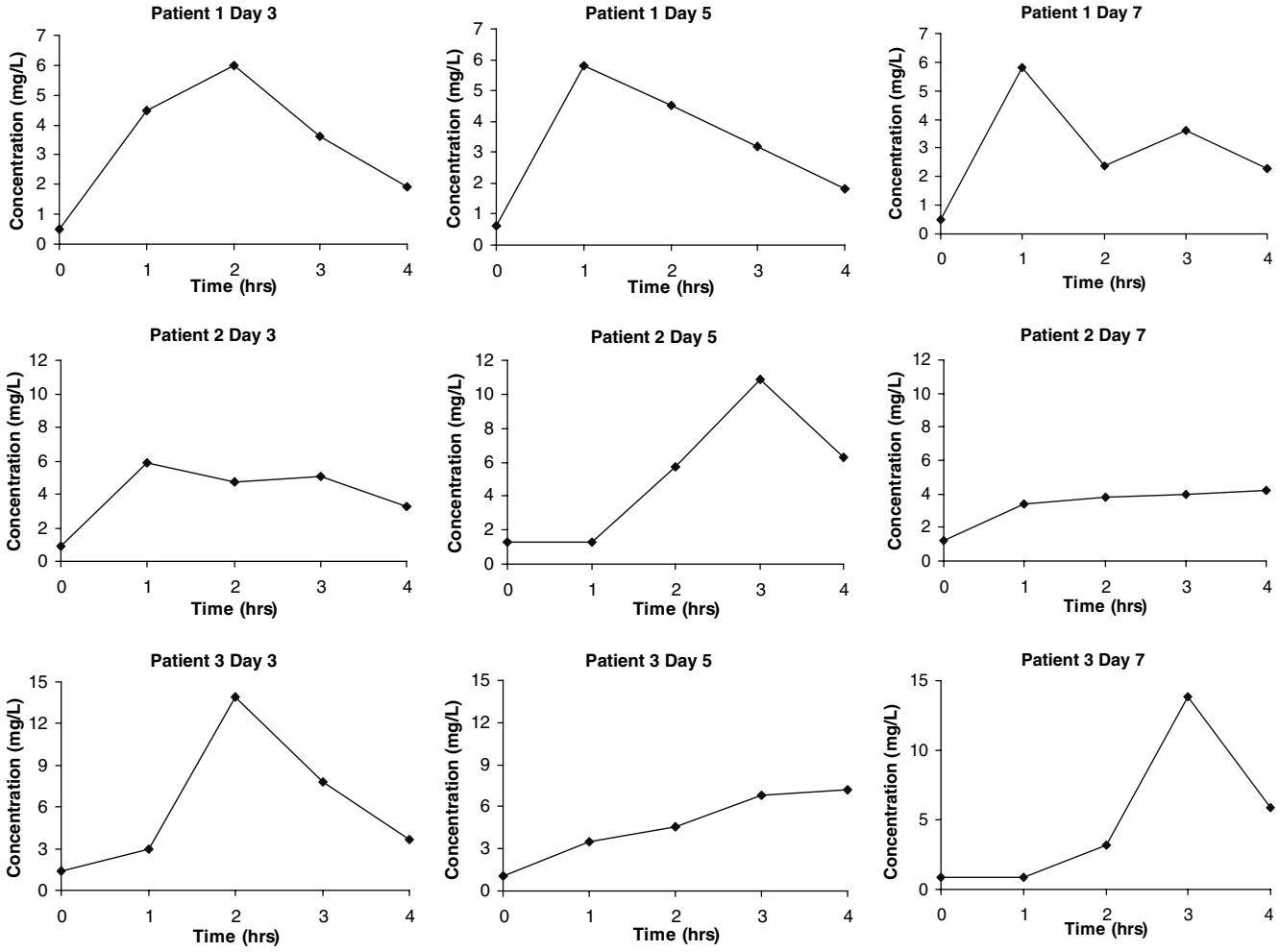

Fig. 2 Concentration–time profiles of mycophenolic acid in three patients on three separate occasions (days 3, 5 and 7)

for describing the data. Inclusion of between-subject variability on the central (V1/F) and peripheral (V2/F) volume compartments could not be supported by the data (same or worse OF) with values having a high standard error. Use of a lag time to describe drug absorption did not improve the model OF.

Consideration of between-occasion variability in CL/F further reduced the model OF, but inclusion of between-occasion variability in V1/F, V2/F, k_a or Q/F did not result in further model improvement. Consideration of enterohepatic circulation could not produce feasible parameter values for the fraction of MPA excreted into the bile or time of gallbladder emptying.

Thus, at this stage, the best base model was a bi-exponential elimination model with first-order absorption estimating apparent clearance (CL/F), central (V1/F) and peripheral (V2/F) compartment apparent volumes of distribution, the absorption rate constant (k_a) and inter-compartmental clearance (Q/F), with between-subject variability on CL/F, k_a and Q/F, and between-occasion variability on CL/F. This model had a proportional residual random error and a diagonal variance–covariance matrix. It remained stable with changing starting estimates and number of significant digits. This basic model yielded a typical (SE%)

Table 2 Summary of base, final and albumin models for CL/F. *ALB* serum albumin concentration (g/l), *FKCY* co-medication type (FKCY = 0 if patient taking cyclosporine, FKCY = 1 if patient taking tacrolimus)

Model	Objective function value
Base model	
$CL/F = \theta_1$ (l/h)	3,017.5
Final model	
$CL/F = \theta_1 \times (1 + \theta_2 \times (ALB - 26)) \times (1 + \theta_3 \times FKCY)$ (l/h)	2978.8
Forward stepwise inclusion	
1. $CL/F = \theta_1$	3,017.5
2. $CL/F = \theta_1 \times (1 + \theta_2 \times (ALB - 26))$ (l/h)	2,989.5
3. $CL/F = \theta_1 \times (1 + \theta_2 \times (ALB - 26)) \times (1 + \theta_3 \times FKCY)$ (l/h)	2,978.8
Albumin model	
$CL/F = \theta_1$ (l/h) $Y1 = Y2 \times ALB \times D_i$ $D_i = \theta_1 \times e^{\eta_i}$ $Y = (Y1 + Y2) \times e^{\epsilon_{ij}}$	2985.9

population CL/F of 29 l/h (5%), V1/F of 66 l (7%), V2/F of 504 l (20%), k_a of 0.65 h (15%) and Q/F of 31 l/h (11%). Between-subject variability in CL/F, k_a and Q/F was 39% (29%), 109% (22%) and 80% (28%), respectively, and between-occasion variability in CL/F was 36% (14%). Residual random error was 41% (4%) (Table 2).

Covariate analysis

From plots of individual Bayesian parameter estimates against covariates, four covariates were identified as being of interest (Figs. 3 and 4). CL/F increased with reducing serum albumin ($r^2 = 0.15$), increased with increasing patient weight ($r^2 = 0.07$), and was greater in patients on cyclosporine than in those on tacrolimus ($P < 0.0001$). There was also a trend towards increased CL/F with reducing creatinine clearance ($r^2 = 0.03$) ($P = 0.067$).

In a (forward) stepwise modelling building approach, the cumulative inclusion of serum albumin and co-medication type reduced the OF by more than 6.68 with each addition (Table 2). Between-subject variability in CL/F reduced from 39% to 34% with consideration of serum albumin and to 32% with the inclusion of both serum albumin and co-medication type, without increasing variability in other parameter estimates. Inclusion of patient weight and/or creatinine clearance in this covariate model failed to cause a further significant OF drop. Furthermore, the influences of patient weight and creatinine clearance were not as well characterised as those of serum albumin and co-medication type, with standard errors associated with these two covariates of 48% and 42%, respectively.

Finally, in the (backward) elimination step, removal of serum albumin and co-medication type increased the OF by more than 6.68 when they were omitted individually from the model. Consideration of these two

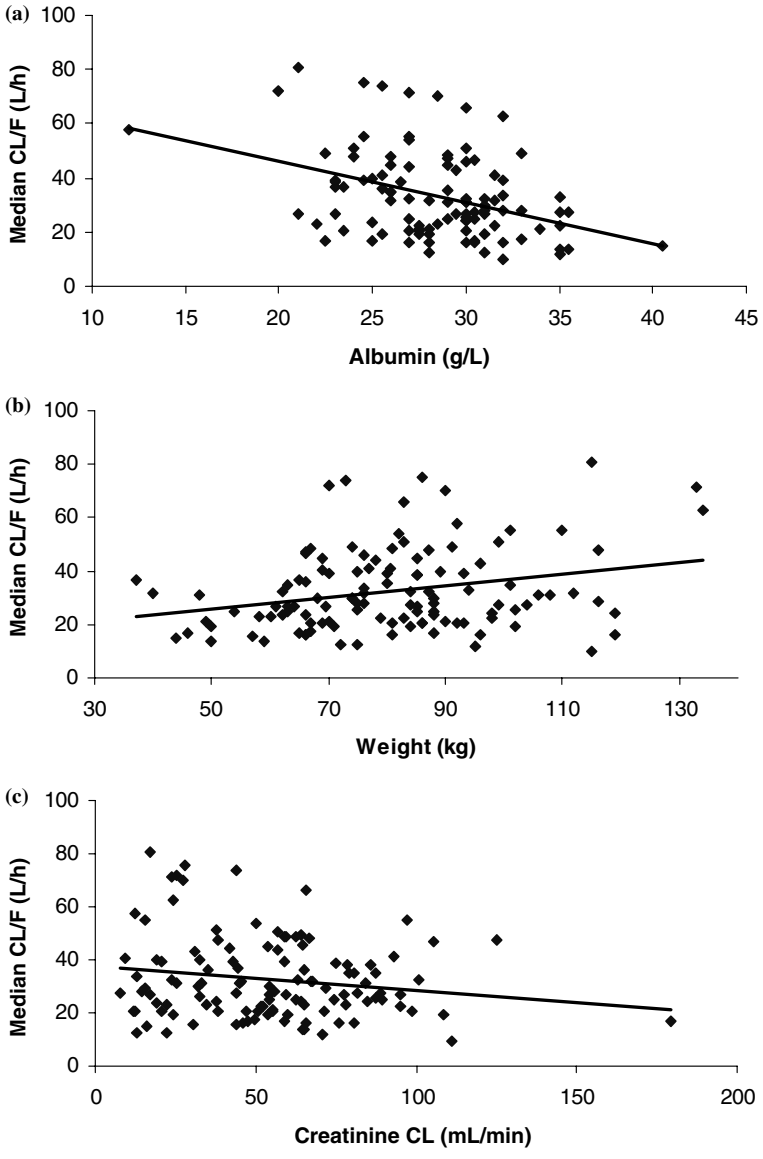

Fig. 3 Scatter plots of empirical Bayesian estimates (median for each individual) versus covariates: (**a**) CL/F versus albumin (**b**) CL/F versus weight and (**c**) CL/F versus creatinine clearance

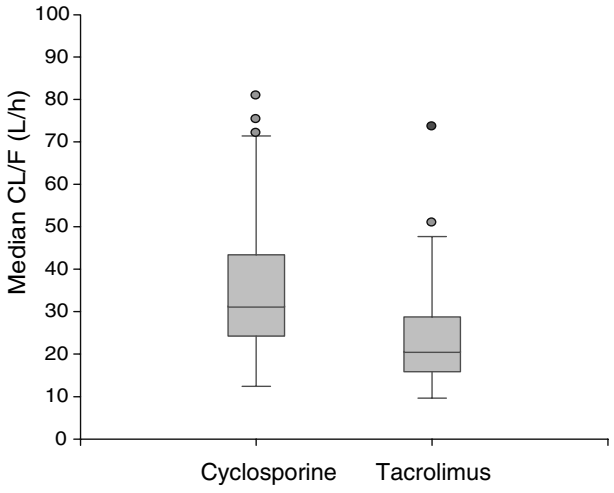

Fig. 4 Empirical Bayesian CL/F estimate (median for each individual) versus co-medication (cyclosporine or tacrolimus)

covariates also improved plots of model-predicted versus observed concentration and residual and weighted residuals versus observed concentration.

Of the subjects, 76% had a serum albumin of less than 31 g/l, making between-subject differences in MPA free fraction likely. As an alternative to modelling the effect of serum albumin as a covariate on CL/F, its incorporation as an albumin-dependent scaling of concentration was also examined. This resulted in an OF drop of 31.6 points (compared with an OF drop of 28.0 points when modelled as a function of CL/F) (Table 2). This model yielded a typical (SE%) population CL/F of 293 l/h (79%), V1/F of 813 l (79%), V2/F of 4740 l (84%), k_a of 0.81 /h (16%), Q/F of 317 l/h (80%) and albumin multiplier of 0.316 (88%). While increased parameter values when considering free drug are not unexpected, these values were estimated with a much higher degree of uncertainty. The model did not appear to be stable when addressed using bootstrap methods with many of the runs terminating unsuccessfully (and therefore were not used for estimating confidence intervals). For those runs that did terminate successfully, the standard deviation of the parameters estimated from the bootstrapped runs (which is equivalent to an asymptotic standard error) were of a similar order of magnitude to those gained from asymptotic computation. This modelling approach was therefore not pursued further.

Thus, the final population model yielded typical population CL/F values of 25.4 l/h (tacrolimus co-therapy) and 34.9 l/h (cyclosporine co-therapy), at the median albumin concentration of 26 g/l, V1/F of 65 l, V2/F of 496 l, k_a of 0.64 h and Q/F of 30% l/h. Between-subject variability in CL/F, k_a and Q/F was 32% (29%), 109% (29%) and 78% (28%), respectively, and between-occasion variability in CL/F was 35% (14%). Residual random error was 41% (4%) (Table 4).

Model evaluation

The parameter values from the final model obtained from the bootstrap analysis were similar to the final model developed using the 117 study patients, with no parameter difference greater than 7%. On comparison of observed and model-predicted (individual) concentrations, the final model was found to be unbiased [mean predicted error (95% confidence interval): 0.11 mg/l (−0.04 to 0.26 mg/l)] but to have a high degree of imprecision [root mean square predicted error (95% confidence interval): 2.80 mg/l (1.74–3.87 mg/l)]. Average bias [mean predicted error (95% confidence interval): 0.12 mg/l (−0.03 to 0.27 mg/l)] and precision [mean predicted error (95% confidence interval): 2.80 mg/l (1.75–3.84 mg/l)] estimated from bootstrap analysis were similar to bias and precision values obtained from the final population model.

Discussion

This study of 117 adult kidney recipients represents the largest population analysis of MPA to date, with investigation of a number of covariate factors and the only analysis focussing solely on the critical early post-transplant phase. Population pharmacokinetic values of CL/F, V1/F, V2/F, k_a and Q/F were quantified during the first week post-transplant, following oral administration of MMF. Additionally, between-subject variability in CL/F, k_a and Q/F and between-occasion variability in CL/F were determined. Total MPA CL/F significantly decreased with increasing serum albumin and was greater in patients receiving cyclosporine than tacrolimus. Values for total MPA CL/F based on various albumin concentrations and whether the patient is receiving cyclosporine or tacrolimus are provided in Table 3. A trend was seen towards increasing CL/F with increasing patient weight and decreasing creatinine clearance, although neither reached statistical significance.

From a clinical perspective, if a total MPA plasma $AUC_{0\text{-}12}$ of 45 mg/h/l was to be targeted, specific values of CL/F from Table 3 could be used as starting estimates for MMF dosage. For a patient with an albumin around 30 g/l receiving tacrolimus, using the starting estimate of CL/F of 21 l/h, a reasonable starting dose would be approximately 1 g twice daily (21×45 mg every 12 h). For a patient receiving cyclosporine, however, with perhaps a lower albumin of around 25 g/l, aiming for the same total plasma AUC would require an estimated starting dose of 1.5 g twice daily (36×45 mg every 12 h). Ideally both free and total mycophenolate concentrations should be measured and appropriate AUC targets developed for free MPA concentration as well as total, but this is not practical advice for the current clinical situation. In this study, the technology [liquid chromatography/tandem mass spectrometry (LC/MS/MS)] was not yet available at the site of MPA

concentration determination for assessment of free (unbound) concentrations. The assay for free MPA is still mainly available at research sites, not at routine laboratories. Serum albumin concentrations are usually measured and available, however. Thus, it is important to be able to use total MPA concentrations and albumin concentrations in an algorithm such as that shown in Table 3 to determine the best dose to achieve specific target AUCs. This study was also not a pharmacodynamic study; therefore, the target AUC to use needs to be derived from other appropriate studies [3, 4, 7, 9, 10].

Population pharmacokinetic analysis is the first step in the development of a Bayesian dosage method for predicting optimal individualised doses for MMF. The number of patients in this study was large enough to provide relevant pharmacokinetic information in the early stages post-renal transplant. For comparison, a summary of results from the two previous population analyses are presented in Table 4 along with parameter estimates from this study [13, 14]. As in these previous studies, this analysis found MPA pharmacokinetics to be better characterised by a bi- rather than single-exponential elimination model. CL/F and apparent volume of distribution estimates in this study were greater than those obtained in 60 stable adult kidney recipients at least 6 months post-transplant [14]. V2/F was also larger than reported in 22 adult kidney recipients in the first month post-transplant [13].

Differences in CL/F determined from total measured MPA plasma concentrations are most likely due to differences in MPA free concentration at different stages post-transplant, although our data do not exclude the possibility that fraction of the dose absorbed may be changing in this critical post-transplant time. The extent of protein binding of MPA in the plasma will influence its volume of distribution, clearance and ability to cause pharmacological and toxic effects. Only free drug can pass from the plasma into the tissue cells, interact at receptor sites and is available for elimination. In future studies, it will be important to measure free concentration of MPA and incorporate this into modelling.

Differences in V2/F between this and the two previous population studies may also be due to increased MPA free concentration during the first week post-transplant. Alternatively, the sparseness of blood sampling in this investigation may have resulted in overestimation of this parameter, as most data were collected before the terminal elimination phase.

It has been widely reported that MPA undergoes enterohepatic circulation, giving rise to a secondary peak concentration between 4 and 12 h after oral administration [1]. It is not surprising this phenomenon could not be successfully modelled in this study given MPA concentrations were only measured between 0 h and 4 h post-dose. Sampling later in the dosing interval would be required to model this more accurately.

Co-medication type (tacrolimus or cyclosporine) did prove to be a significant covariate in our final population model, this may be a result of the different influences cyclosporine and tacrolimus have on enterohepatic recirculation of MPAG. Cyclosporine is believed to inhibit the transportation of MPAG from hepatocytes into the bile resulting in decreased enterohepatic circulation [22]. Some investigators have recommended using a 50% lower dose of MMF in combination with tacrolimus than of cyclosporine [23]. In this study, co-therapy with cyclosporine as opposed to tacrolimus resulted in a mean increase in MPA CL/F of 27%. Dose-normalised MPA trough concentrations were significantly higher in patients receiving tacrolimus than those receiving

Table 3 Mycophenolic acid (MPA) CL/F estimates from the final population model based on albumin concentration and whether the patient is receiving cyclosporine or tacrolimus co-therapy

MPA CL/F (l/h)		
Albumin concentration (g/l)	Co-medication	
	Cyclosporine	Tacrolimus
20	43.7	31.8
25	36.4	26.5
30	29.0	21.1
35	21.7	15.8

Table 4 Comparison of study results with previous population pharmacokinetic studies given as mean values (SE %). Study 1: 60 stable kidney recipients more than 6 months post-transplant [14]. Study 2: 22 kidney recipients during the first month post-transplant [13]. Study 3 (current study): 117 adult kidney recipients during the first week post-transplant. *BSV* between-subject variability, *BOV* between-occasion variability, *RRE* residual random error

Parameter	Units	Study 1	Study 2	Study 3
CL/F	l/h	15.7[a,b] (5%)	27.1[b] (5%)	34.9[b,c]/25.4[c,d]
V1/F	l	36 (19%)	98 (13%)	65 (7%)
V2/F	l	137 (17%)	206 (27%)	496 (20%)
Q/F	l/h	25.9 (36%)	25.7 (13%)	30.7 (10%)
Duration of input (DI)	h	0.69 (7%)	–	–
K_a	/h	–	2.27 (8%)	0.64 (14%)
Lag time	h	–	0.145 (14%)	–
BSV(CL)	%	28	20 (62%)	32 (29%)
BSV(V1)	%	63	56 (58%)	–
BSV(V2)	%	–	151 (98%)	–
BSV(Q)	%	45	–	78 (28%)
BSV(D1)	%	11	–	–
BSV(k_a)	%	–	–	109 (21%)
BSV(lag time)	%	–	–	–
BOV(CL)	%	–	13 (74%)	35 (14%)
BOV(V1)	%	–	95 (29%)	–
BOV(V2)	%	–	51 (90%)	–
BOV(Q)	%	–	–	–
BOV(D1)	%	–	–	–
BOV(k_a)	%	–	184 (21%)	–
BOV(lag time)	%	–	33 (343%)	–
Proportional RRE	%	[e]	35 (61%)	41(4%)
Additive RRE	mg/l	2.04	0.75 (88%)	–

[a] Based on median study weight
[b] Cyclosporine co-therapy
[c] Tacrolimus co-therapy
[d] Based on median study albumin 26 g/l
[e] Not reported

cyclosporine (median 0.00187 mg/l per mg versus 0.001 mg/l per mg, respectively, $n = 269$, $P < 0.00001$). Similar differences in MPA CL/F according to co-therapy have been reported in other studies [23].

Previous studies have reported a minor but generally not clinically significant influence of patient weight on MPA CL/F [1, 13, 14]. In this study, a trend towards increased CL/F with increasing patient weight was evident. While the slope coefficient associated with weight (0.5% increase in CL/F with each 1 kg increase in weight) suggested this covariate was potentially clinically significant, this parameter was estimated with a high degree of uncertainty. Inclusion of patient weight into the population model only resulted in absolute reduction in between-subject variability of 1.3%. As several patients in this study were probably obese, ideal body weight (if it could have been calculated) could have been a better predictor of body size and possibly a more influential covariate.

Markedly impaired renal function has been reported to influence MPA disposition [17, 24, 25]. Patients with acute impairment of renal function have higher MPA CL/F than patients with normal renal function [15, 25], with this returning to the CL/F recorded for people without acute impairment over a 90-day period [25]. Poor renal function can reduce renal excretion of MPAG that is believed to compete with MPA for protein binding sites [15]. In this investigation, a trend towards increased CL/F with decreasing creatinine clearance was evident but never reached statistical significance. Only 13% of patients had a creatinine clearance of less than 20 ml/min on day 7 post-transplant.

Similar to the two previous population studies, our final model displayed large residual variability. Rebound MPA concentrations due to enterohepatic cycling may be one reason for differences in individual predicted and observed concentrations. The predominant reason for poor final model precision, however, is likely to be the erratic nature of individual concentration–time profiles. There was visual evidence of a lag time in drug absorption, a complex absorption process and enterohepatic circulation of MPA in some patients on some occasions. The rate and extent of absorption and enterohepatic circulation of MMF may be dependent on each individual's post-surgical state.

Shum et al. [13] (who reported on multiple blood samples during the first 2 h post-dose) reported 184% between-occasion variability in MMF rate of absorption and 33% variability in absorption lag time. MPA blood concentrations available for this study were determined at $t = 0$ (trough), 1, 2, 3 and 4 h after MMF dosing. MPA concentration at 1 h post-dose was less than or equal to MPA concentration immediately pre-dose in 31 patients (36 occasions) of 171 patients (275 occasions). While there was evidence of a lag time in MMF absorption in some patients in this study, there were not sufficient data in the post-dose absorption period to characterise this parameter sufficiently to cause model improvement.

In theory, changes in MPA free fraction due to changes in albumin should influence all pharmacokinetic parameters except those only involved with drug absorption. Modelling of albumin as an albumin-dependent scaling of concentration, rather than as an effect on CL/F, resulted in parameter values with a high degree of uncertainty. Concentration–time data collected in this study may not have been complete enough that the influence of serum albumin on all pharmacokinetic parameters could be characterised and modelled simultaneously.

The final population model was evaluated, with close agreement between pairs of bootstrapped and final model parameter estimates (all differences $< 7\%$). While model prediction was non-biased, precision was poor, reflecting the large residual variability associated with the final model.

In conclusion, this study has increased the current understanding of MPA pharmacokinetics in the early post-kidney transplant. It included a larger number of patients and samples than in previous investigations and examined several different covariates. MPA displayed complex and somewhat erratic pharmacokinetics over this period. Serum albumin and immunosuppressant co-therapy had the greatest influence on pharmacokinetic variability. Results obtained are important for dosage prediction, with pharmacokinetic parameters now able to be included as starting estimates in Bayesian dosage prediction algorithms and prospectively tested to determine accuracy and precision for achieving target AUCs that minimise rejection and toxicity. Due to the significant influence albumin has on total MPA CL/F early after renal transplant, ideally both free and total mycophenolate concentrations should be measured over this period and appropriate AUC targets developed for free MPA concentration as well as total.

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