

# Population Pharmacokinetics of Mycophenolic Acid in Renal Transplant Recipients

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## Abstract

**Background:** Mycophenolate mofetil is the prodrug of mycophenolic acid (MPA) and is used as an immunosuppressant following renal, heart, lung and liver transplantation. Although MPA plasma concentrations have been shown to correlate with clinical outcome, there is considerable inter- and intrapatient pharmacokinetic variability. Consequently, it is important to study demographic and pathophysiological factors that may explain this variability in pharmacokinetics.

**Objective:** The aim of the study was to develop a population pharmacokinetic model for MPA following oral administration of mycophenolate mofetil, and evaluate relationships between patient factors and pharmacokinetic parameters.

**Patients and methods:** Pharmacokinetic data were obtained from a randomised concentration-controlled trial involving 140 renal transplant patients. Pharmacokinetic profiles were assessed on nine occasions during a 24-week period. Plasma samples for description of full 12-hour concentration-time profiles on the first three sampling days were taken predose and at 0.33, 0.66, 1.25, 2, 6, 8 and 12 hours after oral intake of mycophenolate mofetil. For the remaining six occasions, serial plasma samples were taken according to a limited sampling strategy predose and at 0.33, 0.66, 1.25 and 2 hours after mycophenolate mofetil administration. The resulting 6523 plasma concentration-time data were analysed using nonlinear mixed-effects modelling.

**Results:** The pharmacokinetics of MPA were best described by a two-compartment model with time-lagged first-order absorption. The following population parameters were estimated: absorption rate constant ( $k_a$ )  $4.1\text{h}^{-1}$ , central volume of distribution ( $V_1$ ) 91L, peripheral volume of distribution ( $V_2$ ) 237L, clearance (CL) 33 L/h, intercompartment clearance (Q) 35 L/h and absorption lag time 0.21h. The interpatient variability for  $k_a$ ,  $V_1$ ,  $V_2$  and CL was 111%, 91%, 102% and 31%, respectively; estimates of the intrapatient variability for  $k_a$ ,  $V_1$  and CL were 116%, 53% and 20%, respectively. For MPA clearance, statistically significant correlations were found with creatinine clearance, plasma albumin concentration, sex and cyclosporin daily dose ( $p < 0.001$ ). For  $V_1$ , significant correlations were identified with creatinine clearance and plasma albumin concentration ( $p < 0.001$ ).

**Conclusion:** The developed population pharmacokinetic model adequately describes the pharmacokinetics of MPA in renal transplant recipients. The identified correlations appear to explain part of the observed inter- and intrapatient pharmacokinetic variability. The clinical consequences of the observed correlations remain to be investigated.

## Background

Mycophenolic acid (MPA) is the active component of the prodrug mycophenolate mofetil (CellCept®<sup>1</sup>; Roche Laboratories, Nutley, NJ, USA), and is used to prevent acute rejection in lung, heart, liver and renal transplantation.<sup>[1]</sup> MPA decreases *de novo* purine biosynthesis by inhibiting inosine monophosphate dehydrogenase reversibly and noncompetitively. As a result, the proliferation of T and B lymphocytes is suppressed.<sup>[2]</sup> The pharmacokinetics of MPA after oral administration of a mycophenolate mofetil dose are described by presystemic de-esterification to MPA during a rapid and almost complete absorption phase.<sup>[3]</sup> MPA reaches maximum plasma concentrations ( $C_{max}$ ) within 1 hour in most renal transplant recipients,<sup>[4,5]</sup> and is extensively bound to albumin with an average protein binding of 97.5%.<sup>[6,7]</sup> MPA is mainly metabolised in the liver by uridine diphosphate-glucuronosyltransferase to the inactive 7-*O*-mycophenolic acid glucuronide (MPAG).<sup>[4]</sup>

MPA exhibits considerable inter- and intrapatient pharmacokinetic variability. Its pharmacokinetics are complex since pharmacokinetic parameters change over time following transplantation and appear to be influenced by renal function, albumin concentrations and drug interactions.<sup>[8]</sup> Interestingly, a randomised concentration-controlled trial (RCCT)<sup>[9,10]</sup> led to the suggestion that the incidence of acute rejection and adverse effects is minimised when MPA exposure (area under the plasma concentration-time curve [AUC]) lies between values of 30 and 60 mg • h/L.<sup>[11]</sup> Consequently, therapeutic drug monitoring (TDM) may be useful in reducing and minimising interindividual variability in MPA exposure.<sup>[9,11,12]</sup> At present, TDM is not applied on a

routine basis, but studies assessing the beneficial effects of TDM in mycophenolate mofetil therapy are currently ongoing.

In this study, a clinically applicable population pharmacokinetic model was developed for MPA to elucidate its complex pharmacokinetics. Inter- and intrapatient pharmacokinetic variability was quantified and relationships between pharmacokinetic parameters and patient factors were investigated. Clearly, knowledge derived from the population pharmacokinetics of MPA could improve effective administration of mycophenolate mofetil and may be used to more efficiently apply TDM.

## Patients and Methods

### Patients

Concentration-time data from 140 renal transplant patients who participated in the RCCT were analysed retrospectively. A detailed description of the methods of the RCCT was published previously.<sup>[9,10]</sup> Briefly, its goal was to investigate the relationship between exposure to MPA (AUC, minimum plasma concentration [ $C_{min}$ ],  $C_{max}$ ) and outcome (incidence of acute rejection, adverse effects).

Patients aged 18 years or older were randomly assigned to three MPA AUC target groups: low (AUC 16.1 mg • h/L), intermediate (AUC 32.2 mg • h/L) or high (AUC 60.6 mg • h/L). Starting mycophenolate mofetil doses were 450mg twice daily for the low AUC target group, 950mg twice daily for the intermediate AUC target group and 1700mg twice daily for the high AUC target group. All patients received cyclosporin and corticosteroids as concomitant immunosuppressive therapy according to routine practice.

**1** The use of trade names is for product identification purposes only and does not imply endorsement.

### Sampling Procedure

Concentration-time samples were collected on nine occasions, on days 3, 7, 11, 21, 28, 56, 84, 112 and 140 after transplantation. Plasma samples for description of full 12-hour concentration-time profiles on the first three sampling days were taken predose and at 0.33, 0.66, 1.25, 2, 6, 8 and 12 hours after oral intake of mycophenolate mofetil. For the remaining six occasions, serial plasma samples were taken according to a limited sampling strategy predose and at 0.33, 0.66, 1.25 and 2 hours after of mycophenolate mofetil administration.<sup>[10]</sup> Mycophenolate mofetil dosages were adjusted according to the MPA AUC assessments, targeting at the aforementioned AUC values for the three groups. Patients were required to fast overnight prior to dose administration and for the first 2 hours of the profile after taking the medication. MPA was measured using a validated high-performance liquid chromatography method described elsewhere.<sup>[13]</sup> The lower limit of quantification was 0.1 mg/L. On each sampling day, routine laboratory tests were performed. Graft function was closely monitored; delayed graft function was defined as the need for dialysis in the first week after transplantation. Patient characteristics and biochemical parameters are summarised in table I.

### Pharmacokinetic Analysis

All data were analysed simultaneously using the nonlinear mixed-effects modelling software program (NONMEM Version V, level 1.1) [GloboMax LLC, Hanover, MD, USA]. Because NONMEM estimated pharmacokinetic parameters for MPA, mycophenolate mofetil doses were converted to the equivalent MPA content by multiplying the mycophenolate mofetil dose by 0.739. A logarithmic transformation (natural logarithm) of the concentration-time data was performed because random effects were not sufficiently distributed around zero without the transformation. The first-order estimation method was used throughout the entire model building process, due to the computational intensity of the model using the first-order conditional estimation method.

Several structural models were tested. Models with one, two or three compartments were evaluated, as well as models with and without lag time. Furthermore, it was evaluated whether absorption was best described as a first- or zero-order process. Pharmacokinetic parameters were estimated in terms of clearance (CL), central and peripheral volume of distribution ( $V_1$ ,  $V_2$ ,  $V_3$ ), and intercompartment clearances ( $Q_2$ ,  $Q_3$ ) [NONMEM code TRANS4]. Since bioavailability (F) could not be quantified, CL, Q and V values of MPA correspond to the ratios  $CL/F$ ,  $Q/F$  and  $V/F$ , respectively. Interpatient variability for all pharmacokinetic parameters was modelled using an exponential error model. For example, MPA clearance for the  $i^{\text{th}}$  individual ( $CL_i$ ) was estimated using equation 1:

$$CL_i = \theta_{\text{pop}} \cdot \exp(\eta_i) \quad (\text{Eq. 1})$$

where  $\theta_{\text{pop}}$  represents the population value for MPA clearance, and  $\eta$  represents the interpatient random effect with mean 0 and variance  $\omega^2$ . Intrapatient variability of the pharmacokinetic parameters was also estimated. When estimating both intra- and interpatient variability for clearance, equation 1 becomes equation 2:

$$CL_{ij} = \theta_{\text{pop}} \cdot \exp(\eta_i + \kappa_j) \quad (\text{Eq. 2})$$

where  $CL_{ij}$  is the clearance of MPA for the  $i^{\text{th}}$  individual on the  $j^{\text{th}}$  occasion, and  $\kappa$  is the intrapatient random effect with mean 0 and variance  $\pi^2$ .<sup>[14]</sup> The difference between the  $k^{\text{th}}$  observed MPA concentration of the  $i^{\text{th}}$  individual ( $C_{\text{obs}ik}$ ) and its corresponding model-predicted MPA concentration ( $C_{\text{pred}ik}$ ) was estimated with an additional error model (equation 3):

$$\ln C_{\text{obs}ik} = \ln C_{\text{pred}ik} + \varepsilon_{ik} \quad (\text{Eq. 3})$$

where  $\varepsilon$  is the residual random error with mean 0 and variance  $\sigma^2$ .

The adequacy of the developed NONMEM models was evaluated using the precision of the parameter estimates and goodness-of-fit plots. Standard errors for the estimated population parameters (both

**Table I.** Patient demographics and biochemical parameters<sup>a</sup>

Characteristics	Day 3	Day 28	Day 140
AUC target group (n)			
low (16.1 mg • h/L)	47	45	41
intermediate (32.2 mg • h/L)	45	41	33
high (60.6 mg • h/L)	48	39	25
Gender (n)			
male	88	79	64
female	52	46	35
Race (n)			
Caucasian	131	117	93
Black	3	2	1
other	6	6	5
Diabetes mellitus (n)	7	6	5
Delayed graft function (n)	23	NA	NA
Age (y)	50 (19–70)	51 (19–70)	50 (19–69)
Bodyweight (kg)	69 (37–104)	66 (38–103)	70 (44–98)
Serum creatinine ( $\mu\text{mol/L}$ ) <sup>b</sup>	211 (62–1232)	132 (62–906)	123 (79–255)
Creatinine clearance (mL/min)	31 (4–142)	50 (7–110)	60 (25–123)
Plasma albumin (g/L) <sup>c</sup>	34 (22–45)	37 (23–50)	40 (32–53)
Serum AST (U/L) <sup>d</sup>	18 (3–289)	12 (3–38)	12 (2–10)
Serum ALT (U/L) <sup>e</sup>	15 (2–653)	13 (3–89)	11 (3–54)
Haemoglobin (g/dL) <sup>f</sup>	9.7 (6.7–13.2)	10.3 (6.8–14.8)	11.8 (8.9–18.8)
Ciclosporin daily dose (mg)	600 (0–1400)	450 (200–1400)	300 (150–700)
Patients not taking ciclosporin (n)	3	0	0
MMF dose (mg) bid			
low AUC target group	450 (450–450)	550 (350–850)	450 (250–950)
intermediate AUC target group	950 (950–950)	1300 (500–1750)	1050 (400–1950)
high AUC target group	1700 (1700–1700)	2200 (1100–2200)	2100 (1200–2200)

a Values are expressed as median (range) except for (n).

b Normal values for serum creatinine: 65–115  $\mu\text{mol/L}$  for males and 55–90  $\mu\text{mol/L}$  for females.

c Normal values for plasma albumin: 35–50 g/L.

d Normal values for serum AST: <41 U/L for males and <31 U/L for females.

e Normal values for serum ALT: <37 U/L for males and <31 U/L for females.

f Normal values for haemoglobin: 13.8–16.9 g/dL for males and 12.1–15.3 g/dL for females.

AUC = area under the plasma concentration-time curve; bid = twice daily; MMF = mycophenolate mofetil; n = number of patients; NA = not applicable.

for pharmacokinetic parameters and for random effects) were generated in NONMEM via the covariance option. Goodness-of-fit can be demonstrated in plots of observed concentrations versus model-predicted or Bayesian-predicted concentrations or plots of weighted residuals (WRES) versus time.<sup>[15]</sup> Bayesian-predicted concentrations were obtained with the *posthoc* option in NONMEM. Goodness-of-fit plots were generated in Xpose version 3.010, an S-PLUS based (version 6.1, professional edition, first

release; Insightful Corp., Seattle, WA, USA) modeling aid.<sup>[16]</sup>

#### Covariate Analysis

To explain inter- and intrapatient pharmacokinetic variability, relationships were investigated between pharmacokinetic parameters and patient characteristics. Covariates tested were patient age, sex, race, weight, diabetic status,<sup>[17]</sup> creatinine clearance (CLCR), plasma albumin concentration, liver en-

zymes (AST, ALT), bilirubin, haemoglobin, mycophenolate mofetil dose and ciclosporin daily dose. Creatinine clearance was calculated using the Cockcroft and Gault formula.<sup>[18]</sup> For categorical variables, all data were available. For continuous variables, on average 9% (range 0–16.8%) of data were missing. For the covariates plasma albumin concentration, AST, ALT and bilirubin, more than 10% of data were missing. A missing value for an individual was replaced by the nearest available value in time for that individual. If there was not an available value within 1 month, the missing value was replaced by the corresponding median of the total population.

Individual Bayesian estimates of the pharmacokinetic parameters were generated and relationships between individual parameters and the covariates were visually inspected and investigated in NONMEM. A two-stage approach was used:

1. In the first step, all different covariates were introduced in the structural model separately and tested for their significance. Covariates were introduced in a multiplicative way. Categorical variables, such as sex, were modelled as shown in equation 4:

$$CL_{ij} = \theta_{pop} \cdot \theta^{\text{gender}}$$

(Eq. 4)

where  $\theta^{\text{gender}}$  is the fractional change in clearance in males (in females gender = 0 and in males gender = 1). Continuous variables, such as weight (WT), were modelled around the median value in the population (equation 5):

$$CL_{ij} = \theta_{pop} \cdot (WT/68)^{\theta_{WT}}$$

(Eq. 5)

where the median weight of the total population is 68kg and  $\theta_{WT}$  is an exponential.

Whether inclusion of a covariate significantly improved the fit was determined by two criteria. Firstly, the likelihood ratio test. For two hierarchical models, the difference between the minimum objective function value (OFV), produced by NONMEM for both models, is approximately  $\chi^2$  distributed with degrees of freedom equal to the difference in

the number of estimated parameters in the two models. The model with the lower OFV has a better goodness-of-fit. A p-value of 0.001 was considered to be statistically significant, which corresponds with a decrease in OFV of 10.8 units with one degree of freedom.<sup>[19]</sup> Secondly, a reduction in unexplained inter- and intrapatient variability was evaluated as a criterion for covariate inclusion.

2. In the second step, all covariates selected during the first step were included in an intermediate model. Covariates were excluded separately from the intermediate model (backward elimination). If the elimination of a covariate caused an increase in the OFV of at least 10.8 units ( $p < 0.001$ ; one degree of freedom), the covariate remained in the model. The result of the backward elimination procedure is the final model.

### Model Validation

The stability and performance of the final model were checked with an internal validation of the final model, using the bootstrap resample technique.<sup>[20]</sup> During a bootstrap procedure, approximately 65% of the original data are resampled with replacement, which produces different combinations of datasets. The final model is fitted to each artificial sample to obtain estimates for all parameters (fixed and random effects). The validation of the final model was performed using the bootstrap option in the software package Wings for NONMEM (<http://wfn.sourceforge.net>).

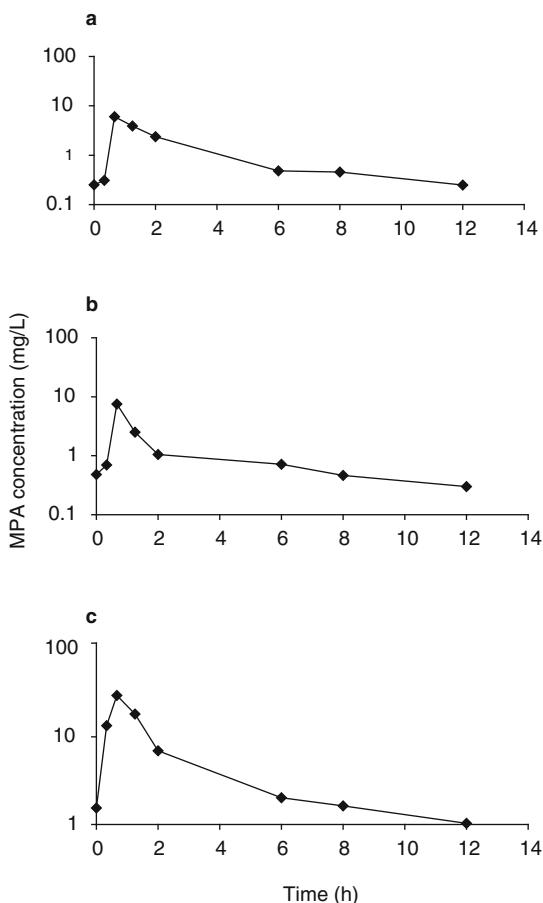
### Statistical Analysis

All statistical analysis, other than the evaluation of the model, were performed with the software package SPSS 10.1 for Windows (SPSS Inc., Chicago, IL, USA). For the comparisons between groups, the Mann-Whitney U test was used. For paired data analysis, the Wilcoxon signed rank test was applied. A p-value  $<0.01$  was considered to be significant given the large sample size.

## Results

### Structural Model

A total of 6523 plasma concentration-time data obtained from 140 renal transplant recipients were analysed. Figure 1 shows characteristic pharmacokinetic profiles of MPA after oral mycophenolate mofetil administration, with a rapid increase in MPA concentration during the absorption phase, often preceded by a lag time, followed by a distribution and an elimination phase. The data were fitted



**Fig. 1.** Characteristic concentration-time profiles of mycophenolic acid (MPA) for the: (a) low area under the plasma concentration-time curve (AUC) [16.1 mg · h/L] target group; (b) intermediate AUC (32.2 mg · h/L) target group; and (c) high AUC (60.6 mg · h/L) target group.

to several structural models. The data fitted a two-compartment model adequately. In a plot of WRES versus time, the residuals were randomly distributed around the axis WRES = 0 and no trends were observed. Adding an extra peripheral compartment yielded a significantly lower OFV, but estimates for peripheral volumes of distribution became unrealistic with values of >10 000L. Consequently, the three-compartment model was rejected. The absorption phase was best described by a first-order absorption rate constant as this gave significantly better fit compared with a zero-order absorption rate constant. Although the likelihood ratio test cannot be applied in this situation, the OFV was 115.9 units lower with first-order absorption. Introduction of an absorption lag time in the model further improved the fit significantly; the OFV decreased by 410.0 units.

Interpatient variability could be estimated for the first-order absorption rate constant ( $k_a$ ), central volume of distribution ( $V_1$ ), peripheral volume of distribution ( $V_2$ ) and clearance; corresponding values were 136%, 126%, 114% and 35%, respectively. The OFV of this model was 1613.9 units. Introduction of interpatient variability on lag time did not improve the model and produced imprecise values for this random effect, therefore it was not included in the model.

Introduction of intrapatient variability for CL,  $k_a$  and  $V_1$  greatly improved the fit of the model. Corresponding values were 30%, 117% and 69%, respectively, and the OFV decreased from 1613.9 to 378.1 units. The estimated population pharmacokinetic parameters of the structural model are summarised in table II and goodness-of-fit plots are given in figure 2. The goodness-of-fit plots in figure 2a and 2b show a symmetrical and random pattern around the line of identity. However, plots of observed concentrations versus model-predicted concentrations on the sampling time points 0.67 hours and 1.25 hours per AUC target group (not shown) provide evidence of a small underestimation of  $C_{max}$ . Because this minor amount of model misspecification is within acceptable limits (99.4% of WRES fall between -3 and 3 and no structural deviations from

**Table II.** Estimates for the structural and final model with their coefficients of variation (CV)

Parameter	Structural model OFV = 378.1 units [estimate (CV%)]	Final model OFV = -338.8 units [estimate (CV%)]	1000 bootstrap replicates [mean estimate (CV%)]
<b>Pharmacokinetic parameters</b>			
$k_a$ ( $\text{h}^{-1}$ )	3.8 (6.2)	4.1 (6.8)	4.1 (7.9)
$V_1$ (L)	98 (9.2)	91 (7.2)	92 (7.2)
$V_2$ (L)	302 (8.4)	237 (10)	239 (11)
$CL$ (L/h)	31 (5.1)	33 (5.4)	33 (5.5)
$Q$ (L/h)	44 (7.9)	35 (5.3)	35 (6.7)
$t_{\text{lag}}$ (h)	0.21 (1.5)	0.21 (1.3)	0.21 (8.7)
<b>Intrapatient variability</b>			
$\eta k_a$ (%)	136 (14)	111 (15)	115 (18)
$\eta V_1$ (%)	126 (15)	91 (13)	91 (8.9)
$\eta V_2$ (%)	114 (26)	102 (25)	103 (13)
$\eta CL$ (%)	35 (15)	31 (15)	31 (7.8)
<b>Intrapatient variability</b>			
$\kappa k_a$ (%)	117 (13)	116 (11)	117 (5.7)
$\kappa V_1$ (%)	69 (14)	53 (17)	53 (11)
$\kappa CL$ (%)	30 (8.8)	20 (11)	20 (5.5)
<b>Residual variability</b>			
Additive error ( $\sigma$ )	0.45 (2.4)	0.45 (2.3)	0.44 (2.4)
<b>Covariates</b>			
$V_1 \theta CL_{\text{CR}}$	NA	-0.62 (16)	-0.62 (16)
$V_1 \theta Alb$	NA	-1.13 (23)	-1.15 (23)
$CL \theta$ gender	NA	1.11 (4.3)	1.10 (4.4)
$CL \theta CL_{\text{CR}}$	NA	-0.12 (30)	-0.12 (31)
$CL \theta Alb$	NA	-1.07 (11)	-1.06 (11)
$CL \theta$ cyclosporin dose	NA	0.31 (11)	0.32 (11)

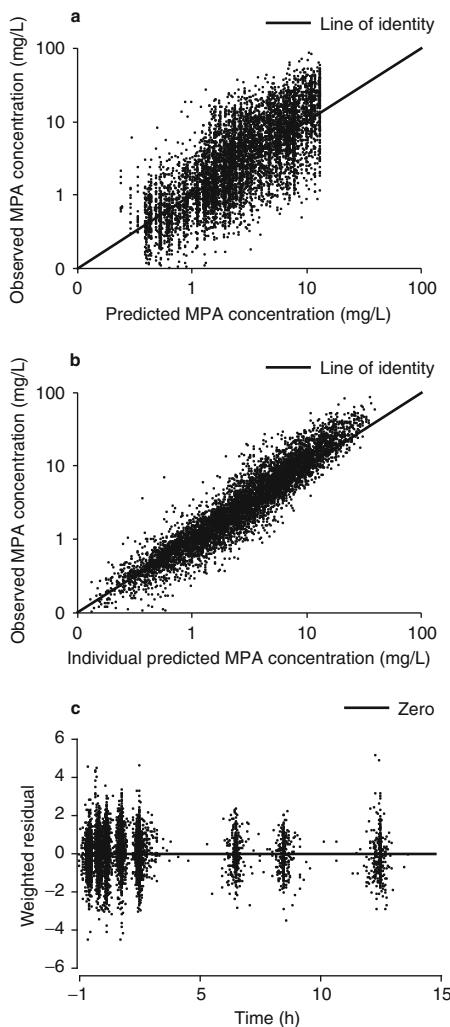
$\theta$  = estimate of covariate; **Alb** = plasma albumin concentration; **CL** = clearance; **CL<sub>CR</sub>** = creatinine clearance;  **$k_a$**  = first-order absorption rate constant; **NA** = not applicable; **OFV** = minimum objective function value; **Q** = intercompartment clearance;  **$t_{\text{lag}}$**  = lag time;  **$V_1$**  = central volume of distribution,  **$V_2$**  = peripheral volume of distribution.

the line  $WRES = 0$  occur [figure 2c]), the model is believed to describe the data adequately.

#### Covariate Analysis

The analysis of the relationship between patient factors and pharmacokinetic parameters produced an intermediate model with the following correlations:  $CL_{\text{CR}}$  and mycophenolate mofetil dose for  $k_a$ ; diabetes mellitus,  $CL_{\text{CR}}$ , plasma albumin concentration and mycophenolate mofetil dose for  $V_1$ ; diabetes, sex,  $CL_{\text{CR}}$ , plasma albumin concentration, cyclosporin daily dose, weight and ALT for  $CL$ ; and plasma albumin concentration for  $V_2$ . During the backward elimination procedure it appeared that  $CL_{\text{CR}}$  and mycophenolate mofetil dose for  $k_a$ , diabe-

tes for  $V_1$ , diabetes and weight for  $CL$ , and plasma albumin concentration for  $V_2$  did not result in a significant increase of OFV when excluded from the intermediate model. These relationships were therefore not incorporated in the final model. All other variables caused a significant rise of OFV when eliminated from the model. Plots of mycophenolate mofetil dose versus  $V_1$  and ALT versus  $CL$  both showed positive correlations. Both were excluded from the model because no pharmacologically sound explanation could be given for the relationship between ALT and  $CL$ , and because the relationship between mycophenolate mofetil dose and  $V_1$  was very weak, albeit statistically significant, and without clinical significance. Population  $V_1$  and  $CL$



**Fig. 2.** Goodness-of-fit plots for the structural model: (a) model-predicted mycophenolic acid (MPA) concentration vs observed MPA concentration; (b) individual Bayesian-predicted MPA concentration vs observed MPA concentration; and (c) sample time vs weighted residuals.

(table II) were described by the following equations in the final model (equations 6 and 7):

$$V_1 = 91 \bullet (\text{CL}_{\text{CR}}/48)^{-0.62} \bullet (\text{Alb}/30)^{-1.13} \quad (\text{Eq. 6})$$

$$\text{CL} = 32.5 \bullet (\text{CL}_{\text{CR}}/48)^{-0.12} \bullet (\text{Alb}/30)^{-1.07} \bullet (\text{CsA}/450)^{0.31} \bullet 1.11^{\text{gender}} \quad (\text{Eq. 7})$$

From these equations, it appears that  $V_1$  typically increases from 80 to 242L when  $\text{CL}_{\text{CR}}$  falls from 60 to 10 mL/min, assuming the plasma albumin concentration (Alb) is 30 g/L. When the plasma albumin concentration increases from 30 to 50 g/L,  $V_1$  decreases from 92 to 53L. Based on equation 7, it appears that males have an 11% higher MPA clearance than females. Furthermore, a reduction in  $\text{CL}_{\text{CR}}$  from 60 to 10 mL/min, with a cyclosporine daily dose (CsA) of 450mg and plasma albumin concentration of 30 g/L, causes an increase in MPA clearance from 32 L/h to 39 L/h (figure 3a). When the plasma albumin concentration rises from 30 to 50 g/L, MPA clearance decreases from 33 to 18 L/h (figure 3b). A cyclosporine dose change from 1000 mg/day to 700 mg/day produces a decrease of MPA clearance from 41 to 33 L/h (figure 3c).

The inclusion of these covariates in the final model explained both the inter- and intrapatient variability for  $V_1$  and CL when compared with the structural model (table II). The estimate for interpatient variability for  $V_1$  decreased from 126% to 91%, and for CL from 35% to 31%. The estimated intrapatient variability was reduced from 69% to 53% for  $V_1$  and from 30% to 20% for CL. All parameters were estimated with an acceptable coefficient of variation of  $\leq 30\%$ . The goodness-of-fit plots of the final model are presented in figure 4.

#### Model Validation

The results of 1000 bootstrap replicates are summarised in table II. The mean estimates resulting from the bootstrap procedure are very similar to the population estimates of the final model. This means that the estimates for the fixed and random effects in the final model are accurate and that the model is stable.

#### Analysis of the Influence of Renal Function on the Decrease of Mycophenolic Acid Clearance

The covariate analysis revealed that renal function significantly correlates with MPA clearance. To investigate whether this effect plays a role in the described decrease in clearance for MPA during the

first weeks after transplantation,<sup>[8]</sup> the study population was divided into two groups: those with CL<sub>CR</sub> <25 mL/min ( $n = 56$ ) and those with CL<sub>CR</sub> >25 mL/min ( $n = 84$ ) on day 3 after transplantation. The cut-off point of CL<sub>CR</sub> 25 mL/min was based on visual inspection of the plotted relationship between CL<sub>CR</sub> and MPA clearance in figure 3a. The group with CL<sub>CR</sub> <25 mL/min contained 21 of the 23 patients who experienced delayed graft function. The course of the Bayesian estimate of clearance over time was studied for both groups.

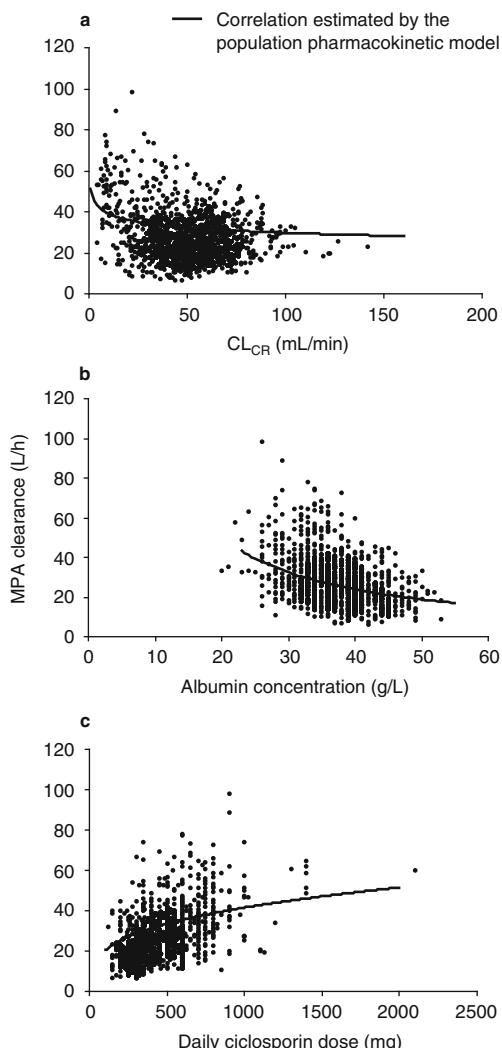
The results are shown in figure 5. Renal transplant patients with CL<sub>CR</sub> <25 mL/min showed a significantly higher mean MPA clearance on days 3 (34% higher;  $p < 0.0001$ ), 7 (22% higher;  $p = 0.005$ ) and 21 (22% higher;  $p = 0.006$ ) after transplantation compared with patients with CL<sub>CR</sub> >25 mL/min. Also, on days 11 and 28 after transplantation, MPA clearance was higher in patients with CL<sub>CR</sub> <25 mL/min (21% higher on day 11;  $p = 0.014$ , and 15% higher on day 28;  $p = 0.026$ ), although not with a significance level of  $p < 0.01$ . Both groups experienced a significant decrease in MPA clearance during the first 28 days after transplantation ( $p < 0.0001$  for both groups). Patients with a CL<sub>CR</sub> <25 mL/min on day 3 had a significantly larger fall in clearance during the first 28 days compared with patients with a CL<sub>CR</sub> >25 mL/min ( $10 \pm 9.6$  vs  $5.2 \pm 8.1$  L/h;  $p = 0.003$ ).

## Discussion

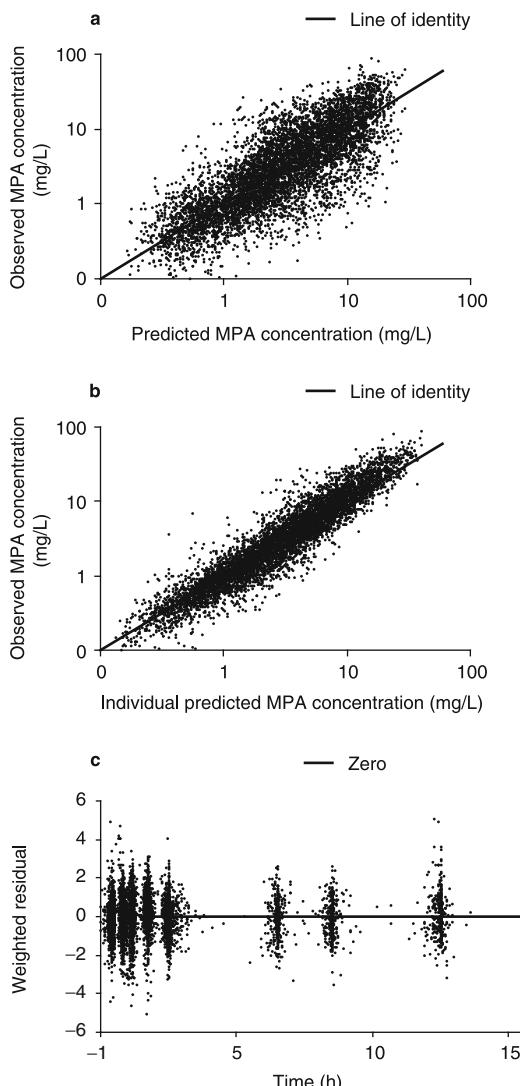
**The pharmacokinetics of MPA following oral administration of the prodrug mycophenolate mofetil exhibit considerable inter- and intrapatient variability.** This study focused on developing a clinically applicable population pharmacokinetic model for MPA to quantify inter-and intrapatient variability and analyse relationships between pharmacokinetic parameters and patient demographics and biochemical factors with the aim of explaining the observed variability.

The pharmacokinetics of MPA in 140 renal transplant patients were described with a two-compartment model with a time-lagged first-order absorption rate. Plots of observed concentrations versus

model-predicted or Bayesian-predicted concentrations demonstrated an adequate goodness-of-fit. Fixed and random parameters were estimated with acceptable precision and residual variability was small (figure 2 and figure 4). Nevertheless, model diagnostics showed a slight underestimation of maximum MPA concentrations ( $C_{max}$ ). The likely reason for this underestimation is that with orally administrated drugs, conventional compartmental



**Fig. 3.** Correlations for mycophenolic acid (MPA) clearance with: (a) renal function (creatinine clearance [CL<sub>CR</sub>]); (b) plasma albumin concentration; and (c) daily cyclosporine dose, as identified in the final model.



**Fig. 4.** Goodness-of-fit plots for the final model: (a) predicted mycophenolic acid (MPA) concentration vs observed MPA concentration; (b) individual Bayesian-predicted MPA concentration vs observed MPA concentration; and (c) sample time vs weighted residuals.

models with a lag time and first- or zero-order absorption rate constant are often not able to accurately predict a rapid initial increase in plasma concentration.<sup>[21]</sup> More complex and mechanistic models may be necessary to accurately describe the absorption phase.<sup>[21]</sup> Since the underestimation of  $C_{max}$  was small and within acceptable limits, the

model was believed to be sufficiently accurate to describe MPA pharmacokinetics and its variability. The validity of the derived model was confirmed by the results of the bootstrap procedure yielding similar values for all parameters and their corresponding precision.

The pharmacokinetic model did not include enterohepatic recirculation (EHC) of MPA, although it has been established that up to 60% of a mycophenolate mofetil dose undergoes recirculation in healthy individuals.<sup>[4]</sup> EHC is responsible for the secondary rise in MPA concentration typically occurring approximately 6–12 hours after oral administration of mycophenolate mofetil.<sup>[4]</sup> However, in the plot of WRES versus time, no trends were observed in this time period and WRES values were randomly distributed around the WRES = 0 axis (figure 2c and figure 4c). As a result, it appears that EHC did not influence the pharmacokinetics of MPA to a large extent in the renal transplant population studied.

The developed population pharmacokinetic model demonstrated considerable interpatient variability among the pharmacokinetic parameters. Only a moderate amount of interpatient variability of CL and  $V_1$  could be explained by inclusion of the covariates renal function, plasma albumin concentration and cyclosporin daily dose: 11% and 28%, respectively. Unexplained interpatient variability of clearance was 31%. Interpatient variability has also been analysed in other studies. One study reported the MPA AUC to range from 7.5 to 94.7 mg • h/L during the first weeks after renal transplantation.<sup>[5]</sup> In that same period, a paediatric study found that interpatient MPA AUC could vary between 28% and 37%, while in another study of adult renal transplant recipients, 32–58% interpatient variability was observed.<sup>[22,23]</sup>

In addition to interpatient variability in exposure, considerable intrapatient variability in exposure during the first weeks after transplantation has also been reported in other studies.<sup>[8,23,24]</sup> In the present analysis, 33% of the intrapatient variability in clearance was explained by the included covariates. The

unexplained intrapatient variability for clearance was small, with a value of 20% in the final model.

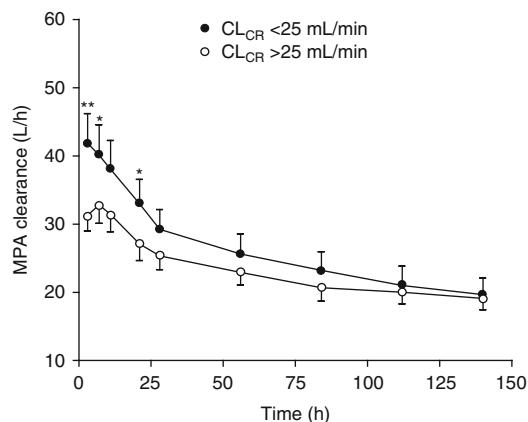
When estimates of inter- and intrapatient variability of pharmacokinetic parameters are available, one might speculate on the usefulness of TDM of mycophenolate mofetil therapy. With the support of TDM, interpatient variability in exposure to the drug is greatly reduced and patients can be targeted to a particular therapeutic window. However, large intrapatient variability reduces the efficacy of TDM since these variations in time cannot be controlled. The present study demonstrated a large interpatient variability and relatively small intrapatient variability for clearance, taking the identified covariates into account. This suggests that TDM may substantially reduce the observed differences in MPA exposure between individuals and may be useful in obtaining AUC values in the range of 30–60 mg · h/L, which are currently proposed.<sup>[11]</sup>

The covariate analysis identified relationships between  $V_1$  and renal function and plasma albumin concentration, and between clearance and renal function, plasma albumin concentration, ciclosporin daily dose and sex. The observation that clearance increases with impaired renal function may be explained by reduced protein binding of MPA caused by uraemia, as well as by MPAG accumulation.<sup>[5]</sup> Both processes may produce an increased free fraction of MPA, which in turn may lead to increased metabolism to MPAG since MPA is believed to have a low to intermediate extraction ratio.<sup>[4]</sup> The dependence of MPA clearance on protein binding has been suggested by several studies<sup>[24,25]</sup> and is confirmed in the present analysis. **However, the correlation between renal function and clearance only appears to be of clinical significance when CLCR is very low (<25 mL/min [figure 3a]).** A decrease in CLCR from 90 to 50 mL/min induces only a modest increase in MPA clearance from 30 to 33 L/h, whereas a fall in CLCR from 50 to 10 mL/min increases MPA clearance from 33 to 39 L/h. The controversy about the influence of renal function on MPA clearance in the literature, where some studies have found a relationship while others have not,<sup>[12,24–27]</sup> may be due to the observation that MPA

clearance is only influenced with severely impaired renal function. The majority of patients with CLCR <25 mL/min suffered from delayed graft function in the first week(s) after transplantation. At this time, patients may have low plasma albumin concentrations, metabolic acidosis and uraemia, resulting in an increase in the MPA free fraction and thus in higher MPA clearance. Subsequently, as the condition of patients improve, normal nutritional status and normal albumin levels are attained. The result may be that free MPA concentrations are not influenced, to a large extent, even when renal function is moderately impaired.

The negative correlation between plasma albumin concentrations and MPA clearance is in accordance with the reported intermediate extraction ratio for MPA, which suggests that its pharmacokinetics depend on the free fraction.<sup>[4,28]</sup> This observed relationship is confirmed by results from other studies. An *in vitro* study showed that increasing albumin concentrations lead to a decreased free MPA fraction.<sup>[7]</sup> This relationship has been confirmed in a paediatric renal transplant population.<sup>[26]</sup> Furthermore, a study using rat livers demonstrated that MPA clearance increased with decreasing albumin levels.<sup>[29]</sup>

Inclusion of the relationships between clearance and both creatinine clearance and plasma albumin



**Fig. 5.** Mean time-course of mycophenolic acid (MPA) clearance for patients with creatinine clearance (CLCR) <25 mL/min ( $n = 56$ ) and CLCR >25 mL/min ( $n = 84$ ) on day 3 after transplantation. Error bars represent 95% confidence interval limits. \*  $p < 0.01$ , \*\*  $p < 0.0001$ .

concentration improved the model. Although creatinine clearance and albumin concentrations are related (albumin concentrations increase with recovering renal function), the correlation was weak in this study ( $r^2 = 0.124$ ) and exclusion of one of these relationships significantly worsened the model, which indicates that both factors independently influence MPA clearance. The dependence of MPA clearance on renal function and albumin levels is further strengthened by the observed relationships between  $V_1$  and renal function and plasma albumin concentrations. These relationships revealed that impaired renal function or decreased plasma albumin concentrations lead to a rise in  $V_1$ , potentially as a consequence of transfer of the increased free MPA quantity into peripheral tissues. This produces a relatively lower total plasma concentration and a higher estimate of  $V_1$ . Unfortunately, from our study it is uncertain how renal function and plasma albumin concentrations affect free MPA concentrations, since free MPA levels were not measured.

Concomitant immunosuppressive medication, such as ciclosporin, has been reported to interact with MPA clearance. Ciclosporin is believed to decrease MPA AUC.<sup>[30,31]</sup> In the present study, an effect of ciclosporin was confirmed by demonstrating a positive correlation between the ciclosporin daily dose and MPA clearance. A logical explanation for this correlation may be the inhibitory effect of ciclosporin on the EHC of MPA.<sup>[32]</sup> Since all patients took ciclosporin as concomitant immunosuppressive therapy, this may, at least in part, explain the adequate goodness-of-fit of the model without including EHC. As EHC seems to be present more profoundly when mycophenolate mofetil is combined with tacrolimus,<sup>[32]</sup> a similar population pharmacokinetic analysis is warranted in a tacrolimus-mycophenolate mofetil-treated renal transplant population.

As a clinical consequence of the covariate analysis, mycophenolate mofetil administration may be optimised, as exposure to MPA may be predicted more accurately when plasma albumin concentrations, ciclosporin daily dose, and renal function are taken into account. Besides, a change in these fac-

tors provides an indication to efficiently apply TDM of MPA. Both mycophenolate mofetil dose optimisation and efficient application of TDM may contribute to a lower risk of acute graft rejection. It is important to note that free MPA concentrations, which were not measured in this study, may be constant in hypoalbuminaemia or severely impaired renal function, through an increased free MPA fraction and an increased MPA clearance. Since free MPA is believed to be the pharmacologically active moiety, mycophenolate mofetil dose increases based on measurement of total MPA would not be warranted in such cases. The influence of the identified covariates on free MPA fraction and free MPA concentrations needs to be further investigated to be able to make sound recommendations regarding TDM of MPA and mycophenolate mofetil dosage adjustments in patients with severe renal impairment or decreased plasma albumin concentrations.

Recently, Shum et al.<sup>[33]</sup> performed a population pharmacokinetic analysis of MPA on the basis of data from 22 patients during the first month after renal transplantation. With a similar structural pharmacokinetic model as in the present study, interpatient variability for both  $k_a$  and  $V_1$  was estimated to be lower, whereas intrapatient variability for  $k_a$  and  $V_1$  was estimated to be higher. For clearance, both estimates for variability were lower with corresponding values of 20% and 13%, and no relationships were assessed between patient factors and clearance. The observed differences with the present study may be explained by the lower number of patients and the shorter follow-up time in the study by Shum et al.<sup>[33]</sup> Another population pharmacokinetic analysis of MPA by Le Guellec et al.<sup>[34]</sup> was performed in stable renal transplant recipients at least 6 months after surgery. MPA clearance was estimated to be 15.7 L/h for a typical patient weighing 64kg with an interpatient variability of 28%. The value for MPA clearance matches well with the value found in this study for the median MPA clearance at day 140 after transplantation, i.e. 18.1 L/h.

With regard to the change of MPA clearance over time, the findings of Shaw et al.<sup>[24]</sup> contrast with the suggestion from several studies<sup>[6,10]</sup> that MPA clear-

ance decreases to the same extent in all renal transplant patients. Shaw et al.<sup>[24]</sup> showed that patients who do not have a well functioning graft immediately after transplantation experience the most pronounced decrease in MPA clearance.<sup>[24]</sup> The negative correlation between MPA clearance and renal function observed in this study confirms that patients with impaired renal function shortly after transplantation will experience a significant change in clearance when renal function improves in the first postoperative weeks. Interestingly, Shaw et al.<sup>[24]</sup> did not find a significant change in MPA clearance in patients with immediate graft function following renal transplantation, whereas our results show a significant change in the group with CLCR >25 mL/min, albeit less pronounced than in patients with CLCR <25 mL/min (figure 5). Other studies<sup>[22,27]</sup> have also found a decrease in MPA clearance regardless of renal function during the first weeks after renal transplantation. This change may be due to changes in the plasma albumin concentration, as well as tapering of the cyclosporin daily dose in the first weeks after transplantation.

## Conclusion

Through the development of a population pharmacokinetic model, more insight was obtained into the pharmacokinetics of MPA after oral intake of mycophenolate mofetil. Inter- and intrapatient variability were quantified and in part explained by correlations between MPA clearance and renal function, plasma albumin concentration and cyclosporin daily dose, and between V<sub>1</sub> and renal function and plasma albumin concentration. The identified correlations offer a possible explanation for the decreasing MPA clearance observed in renal transplant recipients in the first weeks after transplantation. The clinical relevance of changing MPA clearance and V<sub>1</sub> in a situation of altered renal function or plasma albumin concentration remains to be investigated. Nevertheless, the identified relationships may help to apply TDM of MPA more efficiently and to predict MPA concentrations resulting from a certain mycophenolate mofetil dose more accurately, thereby reducing

the variability in MPA concentrations within and between individuals.

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