Explaining Variability in Mycophenolic Acid Exposure to Optimize Mycophenolate Mofetil Dosing: A Population Pharmacokinetic Meta-Analysis of Mycophenolic Acid in Renal Transplant Recipients

Reinier M. van Hest,* Ron A.A. Mathot,* Mark D. Pescovitz,† Robert Gordon,‡ Richard D. Mamelok,§ and Teun van Gelder*‖

*Departments of Hospital Pharmacy and ‖Internal Medicine, Erasmus MC, Rotterdam, The Netherlands; †Department of Surgery, Indiana University Medical Center, Indianapolis, Indiana; ‡Roche Laboratories, Inc., Nutley, New Jersey; and §Mamelok Consulting, Palo Alto, California

Large between- and within-patient variability has been observed in the pharmacokinetics of mycophenolic acid (MPA). However, conflicting results exist about the influence of patient characteristics that explain the variability in MPA exposure. This population pharmacokinetic meta-analysis of MPA in renal transplant recipients was performed to explore whether race, renal function, albumin level, delayed graft function, diabetes, and co-medication are determinants of total MPA exposure. A total of 13,346 MPA concentration-time data points from 468 renal transplant patients who participated in six clinical studies were combined and analyzed retrospectively. Sampling occasions ranged from day 1 after transplantation to 10 yr after transplantation. Concentration-time data were analyzed with nonlinear mixed-effect modeling. Exposure to total MPA, as determined by MPA clearance, significantly increased with increasing renal function, albumin level, and hemoglobin as well as decreasing cyclosporine predose level ($P < 0.001$). These variables could explain 18% of the between-patient and 38% of the within-patient variability in MPA exposure. Differences in MPA exposure between patients with or without delayed graft function or between patients of different races are likely to be caused by the effect of renal function on MPA exposure. Diabetes did not have an effect on MPA exposure. The clinical implication is that a change in renal function or albumin level provides an indication for therapeutic drug monitoring as MPA exposure may be altered. Patients in whom cyclosporine and mycophenolate mofetil are combined may need higher mycophenolate mofetil doses, especially during the early phase after transplantation than currently recommended for optimal MPA exposure.

J Am Soc Nephrol 17: 871–880, 2006. doi: 10.1681/ASN.2005101070

Mycophenolate mofetil (MMF) is an immunosuppressive drug that is used successfully in solid organ transplantation to prevent acute allograft rejection (1,2). MMF is a prodrug of mycophenolic acid (MPA), which exhibits rapid and almost complete absorption from the gut. MPA has extensive plasma albumin binding (98%) and is metabolized by uridine glucuronosyl transferase enzymes into the pharmacologically inactive glucuronide metabolite (MPAG) (3–5). The pharmacokinetics of MPA are characterized further by an enterohepatic recirculation, in which MPAG is excreted into bile and deglucuronidated in the gut back to MPA (3).

Low rates of acute rejection and long-term patient survival have been achieved with MMF when used in a standard dose recommendation of 1 g twice daily for adults. A number of pharmacokinetic studies have shown an increased risk for acute rejection in patients with lower MPA exposure, suggesting that efficacy may improve by adjusting the dose on the basis of plasma concentrations. On the basis of these studies, a target window has been adopted for MPA exposure (area-under-the-curve [AUC] values between 30 and 60 mg/L per h) (6–8). Accumulating evidence suggests that this target is not reached in every patient with the standard MMF dose, with some studies reporting a 10-fold between-patient variability of MPA exposure, changes of exposure over time with a fixed MMF dose, and influence of co-medication (4,9–11). Consequently, individualization of the MMF dose may be necessary to achieve adequate MPA exposure in every patient.

By explaining between-patient variability in MPA pharmacokinetics and identifying the patient characteristics that significantly influence MPA exposure, rational decisions on optimal dosing can be achieved (12). Earlier pharmacokinetic studies have already attempted to correlate MPA exposure to several explanatory factors. For example, some studies found that impaired renal function and low albumin levels result in high total MPA clearance and thus low total MPA exposure (5,9,11,13,14), although this could not be confirmed in all stud-

Received October 17, 2005. Accepted December 6, 2005.

Published online ahead of print. Publication date available at www.jasn.org.

Address correspondence to: Mr. Reinier M. van Hest, Department of Hospital Pharmacy, Clinical Pharmacology Unit, Erasmus MC, Rotterdam, The Netherlands, PO Box 2040, 3000 CA Rotterdam. Phone: +31-10-4633202; Fax: +31-10-4632400; E-mail: r.vanhest@erasmusmc.nl

ISSN: 1046-6673/1703-0871

ies (15–20). Also with regard to the effect of the use of co-medication (3,10,18,21,22), diabetes (23,24), body weight (11,13,17,20), age (5,11,18–20), gender (11,20,23), and race (9,23,25), contrasting results have been obtained. Most of these studies were underpowered, based on MPA predose levels only, and some studies did not control adequately for confounding factors. Consequently, for most variables, it is not clear whether and to what extent they influence MPA exposure and whether individualization of the MMF dose should depend on these variables.

Population pharmacokinetic meta-analysis is known to be very powerful and can estimate reliably the determinants of pharmacokinetic variability, thereby explaining between-patient differences in drug exposure (26,27). An important advantage of a population pharmacokinetic approach is that it allows pharmacokinetic data sets that originate from several studies with different sampling time points to be combined. In this study, a population pharmacokinetic meta-analysis of MPA in renal transplant recipients was performed to explore whether race; age; gender; weight; renal function; albumin level; delayed graft function (DGF); diabetes; and the use of antimicrobial agents, gastric pH modulators, cyclosporine, and corticosteroids can explain variability in MPA exposure between (subgroups of) patients.

Materials and Methods

Studies

Total MPA concentration-time data from 468 renal transplant patients who participated in six different studies were combined and analyzed retrospectively. All data were provided by Roche Laboratories Inc. Details of these studies were published elsewhere previously (6,7,23,28–30). Per study, the number of patients from whom samples were drawn for pharmacokinetic analysis, the MMF starting doses, the occasions of pharmacokinetic assessment after transplantation, the time of sampling after MMF administration, and the concomitant use of immunosuppressive agents are summarized in Table 1.

Data and Definitions

Data on total MPA concentrations, timing of MPA sample drawing, and MMF dosing history from the six studies were pooled in one data set. Data were also collected on patient characteristics, routine laboratory measurements, co-medication, comorbidity such as diabetes, and DGF for every sampling occasion in all patients. Pretransplantation diabetes was defined as the use of antidiabetic drugs within 60 d before transplantation or a medical history of diabetes. DGF was defined as the need for dialysis in the first 2 wk after transplantation. Three categories for race were defined: White, black, and other. The use of co-administered drugs was scored as 1 when the drug was used on the day of pharmacokinetic assessment; otherwise, co-medication was scored as 0. The use of antiviral agents consisted of acyclovir or ganciclovir. Patient characteristics and biochemical parameters are summarized in Table 2.

Pharmacokinetic Analysis

All data were analyzed simultaneously using the nonlinear mixed-effects modeling software program (NONMEM Version V, level 1.1; GloboMax LLC, Ellicott City, MD). NONMEM is a parametric nonlinear multiple measurements regression program that was designed for population pharmacokinetic analyses. This kind of analysis quantifies two types of population pharmacokinetic parameters on the basis of linking dosage, time, and observable patient features to drug concentrations (26,27,31,32). The first type is fixed effect parameters, which quantify mean population pharmacokinetic parameters, or typical relationships between patient features, such as gender or race, and individual pharmacokinetic parameters. The second type is random-effect parameters, which measure between- and within-patient variability of pharmacokinetic parameters (32,33). Using the first-order method in NONMEM, the population pharmacokinetic parameters are calculated by simultaneously fitting all data to a pharmacokinetic model (32). This means that NONMEM appropriately pools data across individuals, which makes the population parameter estimates less dependent on the number of samples per individual, while at the same time it allows easy combination of concentration-time data collected in different studies with different sampling schemes and at different moments after transplantation (27).

A more detailed description of the technical aspects of the methods that are used for pharmacokinetic modeling are reported elsewhere (unpublished observations, Van Hest *et al.*, 2005). Briefly, during the first step of the analysis, a compartmental population pharmacokinetic model was developed describing the pharmacokinetics of MPA and quantifying between- and within-patient variability in MPA pharmacokinetics. Data were logarithmically transformed, and residual variability was modeled additively (27). Individual estimates of the pharmacokinetic parameters were obtained by Bayesian analysis.

The second step was the investigation of relationships between patient factors and individual estimates of the pharmacokinetic parameters. Patient factors that were tested were race; age; gender; weight; albumin level; alanine transferase; bilirubin; alkaline phosphatase; hemoglobin; red blood cell count; DGF; diabetes; cyclosporine dose; cyclosporine predose concentration; MMF dose; corticosteroid dose; the use of antiviral agents, proton pump inhibitors, antacids, H_2-antagonists, and sirolimus; and renal function. With regard to renal function, two estimates were tested: Estimation of creatinine clearance according to the Cockcroft and Gault formula (C&G) (34) and estimation of the GFR with the abbreviated Modification of Diet in Renal Disease (aMDRD) method (35). First, all different variables were tested in the model developed during the first step, in a univariate way. Whether a variable had a significant effect was determined with the likelihood ratio test. $P < 0.001$ was considered to be statistically significant. Second, a multivariate analysis (backward elimination procedure) was done to obtain the final model. The final model was refined by estimating between-patient variability in the relationships between patient factors and pharmacokinetic parameters. This variability parameter takes into account that a change in the value of a variable may not have the same effect on a pharmacokinetic parameter in all individuals (36).

MPA AUC values, normalized to 1000 mg of MMF, were calculated on the basis of the individual estimates for MPA clearance from the final model (equation 1): MPA AUC (mg/L per h) = 1000 mg/MPA clearance (L/h).

Statistical Analyses

Statistical analyses were performed with the software package SPSS 11.5 for Windows (SPSS Inc., Chicago, IL). For comparisons of continuous parameters between groups and within a group over time, repeated measures ANOVA was used. $P < 0.05$ was considered significant.

Results

Data

In total, 13,346 MPA samples that originated from 1894 concentration-time curves that were obtained from 468 renal trans-

Table 1. Description of studies used for the population PK meta-analysis with regard to PK properties[a]

Study (Reference)	No. of Patients	MMF Starting Dose (mg twice daily)	Time of PK Assessment after Transplantation	Time of Sampling after Oral MMF Administration (h)	Concomitant Immunosuppression
Unpublished study	18	1000, 1500, or 1750	Days 1 and 20	Predose, 0.5, 1, 2, 4, 8, and 12	Prednisone Cyclosporine[b]
Sollinger (28), US MMF Study Group (29)	62	1000 or 1500	Days 1 and 5, hospital discharge (range days 6 to 21)	Predose, 0.5, 1, 2, 4, 8, and 12	Prednisone Cyclosporine[c]
Van Gelder *et al.* (6), Hale *et al.* (7)	141	450, 950, or 1700[d]	Days 3, 7, 11, and 21; months 1, 2, 3, 4, and 5	Days 3, 7, and 11; predose, 0.33, 0.67, 1.25, 2, 6, 8, and 12; thereafter: Predose, 0.33, 0.67, 1.25, and 2	Prednisone Cyclosporine[e]
Ekberg *et al.* (30)	44	1000	Days 4 and 7; months 1, 3, and 6	Predose, 0.33, 0.67, 1.25, 2, 3, 4, 6, 8, and 12; day 4 only predose	Prednisone Cyclosporine[e] ($n = 14$) or sirolimus ($n = 30$) Daclizumab
Unpublished study	118	750, 1000	Day 7; months 3, 7, and 12	Predose, 0.33, 0.67, 1.25, 2, 3, 4, 6, 8, and 12	Prednisone Cyclosporine[f] Daclizumab[f]
Pescovitz *et al.* (23)	85	1000, 1250, or 1500	>6 mo (range month 6 to yr 10)	Predose, 0.33, 0.67, 1.25, 2, 3, 4, 6, 8, and 12	Prednisone Cyclosporine[e]

[a]MMF, mycophenolate mofetil; PK, pharmacokinetic.
[b]In the unpublished study, cyclosporine was initiated when creatinine levels dropped below 3 mg/dl.
[c]In the study by Sollinger (28) and by the US MMF Study Group (29), cyclosporine was initiated after the first week.
[d]In the study by Van Gelder *et al.* (6) and by Hale *et al.* (7) MMF dose was based on area-under-the-curve (AUC) measurements to obtain target exposure in three predefined groups (low AUC target group [target AUC 16.1 mg/L per h], intermediate AUC target group [target AUC 32.2 mg/L per h], and high AUC target group [target level 60.6 mg/L per h]). MMF was dispensed as tablets of 250 mg, but to reach target exposure as closely as possible, the dose could be fine-tuned with capsules of 50 mg of MMF.
[e]In the remaining studies (6,7,23,30), cyclosporine was used according to routine practice.
[f]The study submitted for publication was a three-arm study: One arm with standard doses of cyclosporine (predose levels for the first 4 mo of 150 to 300 ng/ml; thereafter, 100 to 200 ng/ml); one arm with low-dose cyclosporine (predose levels of 50 to 100 ng/ml) and standard dose daclizumab; and one arm in which cyclosporine was given in a low dose for the first 3 mo, then cyclosporine was withdrawn over a 3-mo period and standard dose daclizumab.

plant recipients were analyzed. Sampling occasions varied from day 1 to day 3795 (>10 yr) after renal transplantation, and MMF doses ranged from 250 mg twice daily to 2200 mg twice daily. A total of 884 MPA concentration-time curves originated from the first month after transplantation, and 280 pharmacokinetic profiles were taken after the first half year.

Pharmacokinetic Analysis

The model after the first step was a two-compartment model with a lag time that preceded the absorption phase (Figure 1). The results of the uni- and multivariate analyses of relationships between pharmacokinetic parameters and patient factors are summarized in Table 3. The correlations between pharmacokinetic parameters and C&G were statistically stronger as determined with the likelihood ratio test than correlations between pharmacokinetic parameters and aMDRD. For this reason, C&G was used as the measure for renal function and aMDRD was rejected.

After the multivariate analysis, significant relationships were found between C&G, albumin level, hemoglobin and cyclosporine predose level, and MPA clearance (all $P < 0.001$; Table 3). These correlations are reported as relationships with MPA $AUC_{0\ to\ 12}$ (normalized to 1000 mg of MMF, equation 1), as clearance and dose are the only determinants of $AUC_{0\ to\ 12}$. MPA $AUC_{0\ to\ 12}$ was higher when renal function was better: $AUC_{0\ to\ 12}$ was 36 mg/L per h with a C&G of 20 ml/min and 45 mg/L per h when C&G was 65 ml/min (Figure 2A). A higher albumin level correlated with a higher MPA $AUC_{0\ to\ 12}$: 42

Table 2. Patient demographics and biochemical parameters[a]

Characteristics	Days 0 to 4[b]	Month 1	Month 6	Year 1
Gender (*n*)				
male	157	119	87	104
female	89	69	47	67
Race (*n*)				
white	217	168	121	131
black	17	7	4	37
other	12	13	9	3
Diabetes (*n*)	49	17	15	16
DGF (*n*)	34	—	—	—
Use of antacids (*n*)	56	24	0	1
Use of proton pump inhibitors (*n*)	7	13	0	0
Use of H_2-antagonists (*n*)	66	82	3	5
Use of antiviral agents (*n*)	68	8	1	0
Use of sirolimus (*n*)	21	27	17	2
Age (yr)	50 (18 to 72)	50 (19 to 70)	49 (28 to 70)	52 (22 to 73)
Body weight (kg)	71 (37 to 151)	68 (38 to 151)	80 (42 to 151)	75 (49 to 122)
Serum creatinine (μmol/L)	424 (66 to 1379)	128 (53 to 913)	124 (62 to 195)	125 (52 to 221)
Creatinine clearance (ml/min)	19 (4 to 132)	55 (7 to 203)	71 (44 to 132)	64 (34 to 113)
Plasma albumin (g/L)	35 (23 to 51)	35 (26 to 50)	36 (29 to 45)	42 (31 to 48)
Serum ALT (U/L)	17 (2 to 653)	17 (4 to 144)	25 (10 to 128)	20 (11 to 1759)
Serum total bilirubin (mg/dl)	0.5 (0.2 to 3.0)	0.6 (0.1 to 1.9)	0.5 (0.1 to 1.6)	0.7 (0.2 to 3.3)
Serum alkaline phosphatase (U/L)	64 (17 to 870)	86 (25 to 221)	99 (46 to 218)	171 (41 to 347)
Red blood cell count ($\times 10^{12}$/L)	3.2 (1.5 to 4.8)	3.4 (2.1 to 4.9)	4.3 (3.5 to 5.9)	4.4 (3.7 to 9.5)
Hemoglobin (g/dl)	9.7 (4.9 to 17)	11 (6.7 to 15)	12 (9.6 to 18)	13 (7.8 to 18)
Prednisone daily dose (mg)	30 (20 to 1365)	19 (7.5 to 35)	10 (0 to 10)	9.4 (0 to 10)
Cyclosporine daily dose				
in mg	530 (0 to 1000)	350 (0 to 1400)	50 (0 to 200)	138 (0 to 300)
in mg/kg	6.0 (0 to 18)	6.4 (0 to 22)	0.5 (0 to 3.6)	1.8 (0 to 6.6)
Cyclosporine predose level (ng/ml)	171 (0 to 806)	237 (0 to 571)	93 (0 to 316)	155 (0 to 1337)
Patients not using cyclosporine[a]	102	27	17	34
MMF dose				
in mg twice daily	1150 (400 to 2200)	1000 (250 to 2200)	1000 (1000 to 1000)	1000 (250 to 1250)
in mg/kg twice daily	15 (4.8 to 36)	15 (3.9 to 45)	11 (2.2 to 18)	14 (3.8 to 21)

[a]Data are presented as median (range) for four sampling occasions after renal transplantation: Days 0 to 4, month 1, month 6, and year 1. In total, data were collected from 468 renal transplant recipients who participated in six clinical studies. DGF, delayed graft function; ALT, alanine transferase. Normal values for creatinine: 65 to 115 μmol/L for men and 55 to 90 μmol/L for women; for plasma albumin: 35 to 50 g/L; for serum ALT: <41 U/L for men and <31 U/L for women; for serum total bilirubin: <1 mg/dl; for serum alkaline phosphatase: <120 U/L; for red blood cell count: 4.4 to 5.6 $\times 10^{12}$/L for men and 3.9 to 4.9 $\times 10^{12}$/L for women; for hemoglobin: 13.8 to 16.9 g/dl for men and 12.1 to 15.3 mg/dl for women.

[b]For demographic description of the study population during days 0 to 4, one value per variable per patient was used, namely the one measured on the day of PK assessment. Because of different moments of PK assessment after transplantation in the studies, the number of individuals from whom data were available differs for the four presented occasions.

mg/L per h when albumin level was 35 g/L and 48 mg/L per h with an albumin level of 42 g/L (Figure 2B). Furthermore, $AUC_{0\ to\ 12}$ was higher with a hemoglobin of 12.5 mg/dl ($AUC_{0\ to\ 12}$ was 45 mg/L per h) compared with a hemoglobin of 10 mg/dl ($AUC_{0\ to\ 12}$ was 42 mg/L per h; Figure 2C). Finally, a lower cyclosporine predose level correlated with a higher $AUC_{0\ to\ 12}$: 45 mg/L per h with a cyclosporine predose level of 150 ng/ml and 43 mg/L per h with a predose level of 225 ng/ml (Figure 2D). Whereas the separate patient factors had a small to modest effect on MPA $AUC_{0\ to\ 12}$, an almost doubling of $AUC_{0\ to\ 12}$ from 31 to 56 mg/L per h was found when the described effects of renal function, albumin level, hemoglobin, and cyclosporine predose level were combined.

Furthermore, absorption half-life was found to be significantly longer with a lower cyclosporine dose: 0.15 h with a cyclosporine dose of 500 mg and 0.26 h without the use of cyclosporine ($P < 0.001$), indicating that cyclosporine increased the rate of MPA absorption from the gut. Patients who used antacids had a 37% higher central volume of distribution than patients who did not use these agents.

The parameter estimates of the final model are summarized in Table 4. The identified relationships between patient factors

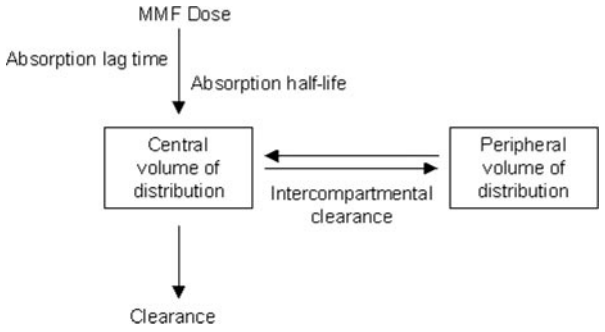

Figure 1. The population pharmacokinetic model that best fit the data, which was a two-compartment model with time-lagged first-order absorption. MMF, mycophenolate mofetil.

and pharmacokinetic parameters after the multivariate analysis (Table 3) explained both between- and within-patient variability in the pharmacokinetics of MPA. A total of 18% of the between-patient variability in clearance and 38% of the within-patient variability in clearance was explained. For absorption half-life, 35% between- and 15% within-patient variability could be explained. For central volume of distribution, 39% between- and 20% within-patient variability was explained. Finally, 42% between- and 47% within-patient variability was explained for intercompartmental clearance. The magnitude of the effect of renal function on MPA clearance and of albumin level on MPA clearance could be very different per individual as illustrated by coefficients of variation for the between-patient variability in these relationships of 66 and 112%, respectively ($P < 0.001$). This indicates that a change of renal function or albumin level may have a significant impact on MPA exposure in one patient, whereas in another patient, the effect may be considerably less.

Effects of Cyclosporine Exposure, DGF, Race, and Diabetes

To illustrate further the influence of the use of cyclosporine on MPA exposure, we compared the course of dose-normalized MPA $AUC_{0\ to\ 12}$ over time after transplantation between patients who had cyclosporine as concomitant immunosuppressive therapy ($n = 144$ on day 0 to 4 posttransplantation) and patients who used an immunosuppressive regimen without cyclosporine ($n = 102$ on day 0 to 4 posttransplantation; Figure 3). Part of this latter group was concurrently treated with sirolimus ($n = 30$). Patients who were exposed to cyclosporine exhibited lower median dose-normalized MPA $AUC_{0\ to\ 12}$ values than patients who were not exposed to cyclosporine during the whole study period, with the exception of the first week. At months 1, 3, and 6 and at year 1 after transplantation, patients who used cyclosporine had a median dose-normalized MPA $AUC_{0\ to\ 12}$ of, respectively, 36, 45, 52, and 56 mg/L per h, and patients without cyclosporine exposure had a median MPA $AUC_{0\ to\ 12}$ of 65, 58, 77, and 72 mg/L per h. Of note, these values also show that MPA exposure increased with time after transplantation.

In the univariate analysis, patients with DGF had a significantly lower median MPA $AUC_{0\ to\ 12}$ compared with those with immediate graft function during the first 4 d after transplantation (23 *versus* 33 mg/L per h, respectively; $P < 0.001$; Figure 4). However, in the multivariate analysis, DGF was no longer significantly correlated with clearance (Table 3), because renal function, as the more broadly defined variable, could explain the lower MPA exposure in patients with DGF: Median C&G was 10 ml/min in patients with DGF *versus* 23 ml/min in patients with immediate graft function during the first 4 d after transplantation ($P < 0.001$). Thereafter, with recovering renal function in patients with DGF (21 ml/min in week 2), the difference in MPA $AUC_{0\ to\ 12}$ between patients with or without DGF decreased: 27 *versus* 33 mg/L per h during days 5 to 8 and 31 *versus* 33 mg/L per h during week 2.

Black renal transplant patients exhibited lower median dose-normalized MPA $AUC_{0\ to\ 12}$ values during the first month after transplantation compared with white patients. $AUC_{0\ to\ 12}$ values on days 0 to 4, days 5 to 8, week 2, and month 1 were 30, 25, 25, and 30 mg/L per h for black patients and 32, 32, 33, and 38 mg/L per h for white patients. Race, however, was not significantly correlated with clearance in the multivariate analysis (Table 3). The difference therefore may be the result of a lower median renal function in black patients during the same occasions (10, 16, 25, and 52 ml/min) compared with white patients (21, 29, 38, and 55 ml/min). The level of renal function over time between both races, however, was not statistically significant ($P = 0.18$). This may be due to the small number of black patients per occasion ($n = 17$, 8, 6, and 7, respectively), resulting in insufficient power to find a statistically significant difference.

In this study, patients with diabetes had a small but significantly increased T_{max} (calculated according to reference [37]) compared with patients without diabetes during the first half year after transplantation. For example, median T_{max} in renal transplant patients with diabetes at 1 mo posttransplantation was 1.1 h and in patients without diabetes was 0.8 h ($P = 0.045$).

Discussion

During standard-dose MMF therapy, the MPA exposure has been reported to vary 10-fold between patients, resulting in a wider range of MPA AUC values than the adopted AUC range of the therapeutic window (4). This suggests that dose individualization may improve outcome. Several studies investigated the determinants of the variability in MPA concentrations, but conflicting results have been obtained (3,5,9–11,13–25). To explore which factors can explain variability in the pharmacokinetics of MPA, we performed a powerful population pharmacokinetic meta-analysis using data from 468 renal transplant recipients. Eight variables that significantly influenced the pharmacokinetics of MPA were identified (Table 3). With these eight variables, 18 to 42% of the between-patient variability and 15 to 47% of the within-patient variability can be explained in the different pharmacokinetic parameters.

Renal function was an important determinant of MPA clearance. MPA clearance decreased with improving renal function. This correlation could explain 35% of within-patient variability, meaning that recovering renal function can explain an important part of the widely known increase of MPA exposure within

Table 3. Relationships between PK parameters and patient factors[a]

PK Parameter	Significantly Correlated Variables after Univariate Analysis[b]	Significantly Correlated Variables after Multivariate Analysis[b]
Absorption half-life	C&G	Cyclosporine dose
	aMDRD	
	Cyclosporine dose	
	Use of H_2-antagonists	
Central volume of distribution	C&G	C&G
	aMDRD	Albumin level
	Albumin level	Use of antacids
	Hemoglobin	
	ALT	
	Cyclosporine dose	
	Use of antacids	
	Use of antiviral agents	
Clearance	C&G	C&G
	aMDRD	Albumin level
	DGF	Hemoglobin
	Albumin level	Cyclosporine predose level
	Hemoglobin	
	Red blood cell count	
	ALT	
	Alkaline phosphatase	
	White race	
	Cyclosporine dose	
	Cyclosporine predose level	
	Corticosteroid dose	
	Use of sirolimus	
	Use of antacids	
	Use of antiviral agents	
	Use of H_2-antagonists	

[a]Relationships between PK parameters and patient factors were tested using the model shown in Figure 1. Relationships first were tested in a univariate way; thereafter, the significantly correlating variables were included in a multivariate analysis (backward elimination procedure) to obtain the final model. C&G, estimation of the creatinine clearance according to Cockcroft and Gault; aMDRD, estimation of the glomerular filtration rate according to the abbreviated Modification of Diet in Renal Disease method.

[b]All relationships between PK parameters and patient factors were significant at the level of $P < 0.001$.

a patient over time (7). The relationship between renal function and MPA clearance also explained why patients with DGF had a higher MPA clearance and consequently a lower MPA $AUC_{0\ to\ 12}$ in the first days after transplantation compared with patients with immediate graft function (Figure 4) (9,38). Patients with DGF had lower MPA exposure as a result of a significantly lower renal function during that period compared with patients without DGF.

A similar effect may apply to race. Black patients showed a trend toward lower dose-normalized MPA $AUC_{0\ to\ 12}$ compared with white patients in the first month after transplantation. Like DGF, this difference may be explained by a lower renal function in black patients, without an additive effect attributable to race. Although speculative, a possible difference in renal function between races and the resulting effect on MPA exposure might have contributed to the observation in a previous study that black patients benefited from MMF over azathioprine only with doses of 1.5 g twice daily, instead of the standard dose of 1 g twice daily (9,25).

The influence of renal function on MPA clearance was not found in every study that investigated the pharmacokinetics of MPA (15,16,18,19). This is explained by the fact that renal function seems to have a clinically relevant effect on MPA clearance only when renal function is <25 ml/min (Figure 2A). Changes in renal function above the 25 ml/min threshold have a small impact on MPA clearance: An improvement of renal function from 65 to 110 ml/min induces a modest decrease of MPA clearance from 22 to 19 L/h (Figure 2A). Studies with low proportions of patients with DGF or impaired graft function postoperatively may have been underpowered to demonstrate the influence of renal function on MPA clearance.

Acidosis, uremia, and accumulation of MPAG, all associated with impaired renal function, will decrease MPA binding to albumin (9). As MPA is supposed to be a restrictively cleared

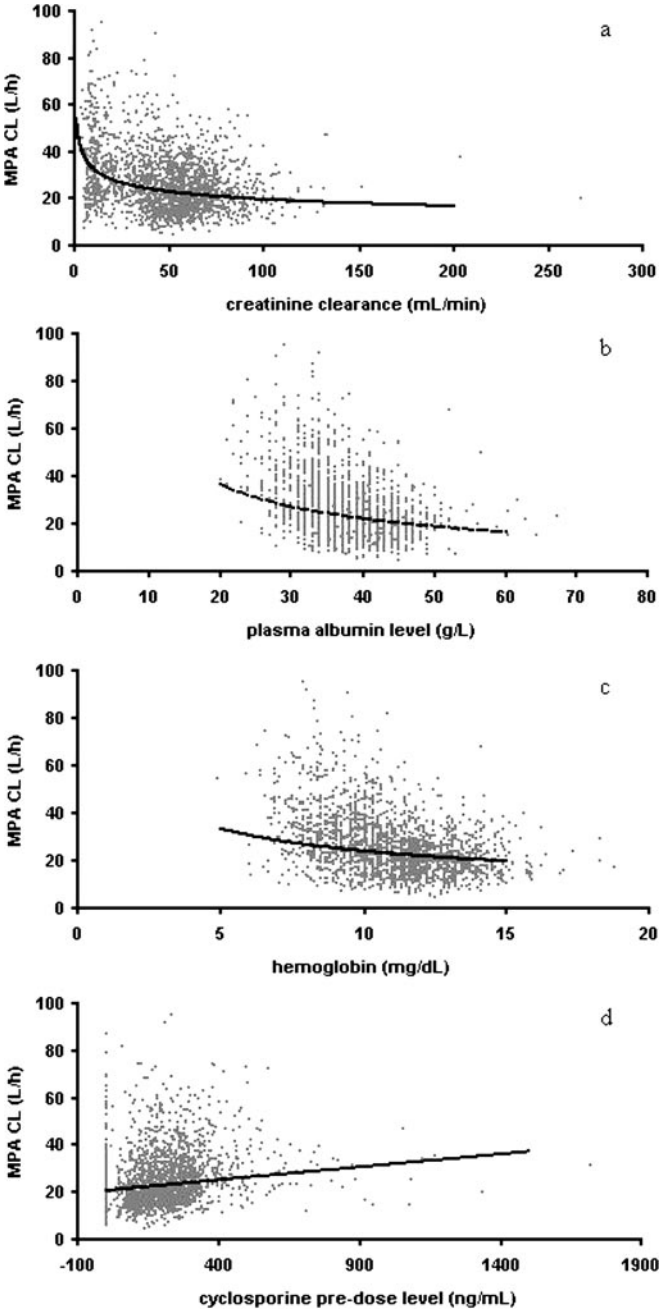

Figure 2. Correlations between mycophenolic acid (MPA) clearance (CL) and renal function (creatinine clearance calculated according to Cockcroft and Gault; A), plasma albumin level (B), hemoglobin (C), and cyclosporine predose level (D). The solid lines represent the correlation estimated by the final population pharmacokinetic model.

drug, an increased free fraction leads to an increase of the amount of MPA that is available for glucuronidation and hence to a higher MPA clearance (9).

The relationship with plasma albumin level and MPA clearance is also explained through MPA free fraction. When the albumin level increases, MPA free fraction may become smaller; consequently, MPA clearance may decrease. The effect that increasing hemoglobin caused a decrease in MPA clearance, which was not found earlier, might also be explained with the same hypothesis. This suggests that MPA binds not only to albumin but also to hemoglobin or red blood cells. Unfortunately, free MPA concentrations were not available in this study to test this hypothesis.

Despite having identified the significant influence of renal function and plasma albumin level on MPA clearance, adjustments of MMF dose cannot be recommended purely on the basis of these factors. The reason is that large between-patient variability was estimated in the effect that renal function and albumin level had on MPA clearance (66 and 112%, respectively). This means that the same change in renal function or albumin level in one patient may result in a clinically relevant change of MPA clearance, whereas in another patient, hardly any effect will be present. Consequently, a change in renal function or albumin level is not in itself an indication for dose adjustment but is merely an indication for therapeutic drug monitoring to check whether the MMF dose needs to be adjusted to get or keep MPA exposure on target. Another reason may be that despite lower total MPA exposure, free MPA concentrations may be unaltered or even elevated in situations of impaired renal function or low albumin levels (9,39). Because free MPA is regarded as the pharmacologically active moiety (40), MMF dose adjustments would not be indicated then. This issue warrants further research before MMF dose can be based on renal function and albumin level.

The observation that MPA clearance is influenced by cyclosporine predose level can be explained by cyclosporine-mediated inhibition of the multidrug resistance protein 2 through which the enterohepatic recirculation of MPA can be disrupted (10). The result is that patients who were treated concurrently with cyclosporine had lower MPA exposure than patients who did not receive cyclosporine during the first year after renal transplantation (Figure 3). This observation is in accordance with observations from other studies in which patients who were treated concurrently with sirolimus (41) or tacrolimus (21) had higher MPA exposure than cyclosporine-treated patients. Furthermore, Figure 3 shows that half of the patients who were treated concurrently with cyclosporine had MPA exposure below the recommended target window in the first week after transplantation. Because optimal MPA exposure early after transplantation is associated with a lower incidence of acute rejection (42), outcome in patients in whom MMF is combined with cyclosporine may be improved with 1500 mg of MMF twice daily instead of the currently recommended 1000 mg twice daily in the immediate posttransplantation phase.

The result from a previous study that the tapering of corticosteroids leads to an increase of MPA concentrations could not be confirmed (22). A positive correlation between the corticosteroid dose and MPA clearance could be identified during the univariate analysis, but this relationship lost its significance in the multivariate analysis. This indicates that the corticosteroid dose is a confounding factor for the relationships between the patient factors and MPA clearance in the final model.

A previous study did not show an effect of diabetes on MPA $AUC_{0\ to\ 12}$ (23). Another study found an increased T_{max}, but only seven patients with diabetes were included (24). This study con-

Table 4. Parameter estimates for the final model with their SE[a]

PK Parameter	Population Estimate (Mean [SE])	Between-Patient Variability (%CV [SE])	Within-Patient Variability (%CV [SE])
Absorption half-life	0.17 h (0.012)	101% (14)	116% (12)
Central volume of distribution	69 L (4.0)	90% (14)	71% (8.3)
Clearance	23 L/h (0.54)	36% (3.4)	21% (2.1)
Peripheral volume of distribution	298 L (23)	—	—
Intercompartmental clearance	34 L/h (2.5)	60% (13)	41% (16)
Absorption lag time	0.24 h (0.0028)	—	—

[a]Parameters were estimated taking into account the effect of cyclosporine dose on absorption half-life, the effect of renal function (creatinine clearance calculated according to C&G), plasma albumin concentration, and the use of antacids on central volume of distribution and the effect of renal function, albumin level, cyclosporine predose level, and hemoglobin on clearance. Estimates for between- and within-patient variability represent the unexplained random variability. No estimate for between- or within-patient variability does not mean that there is no variability in the concerning parameter but that the data do not contain sufficient information to allow reliable estimation of the variability. %CV, coefficient of variation for variability.

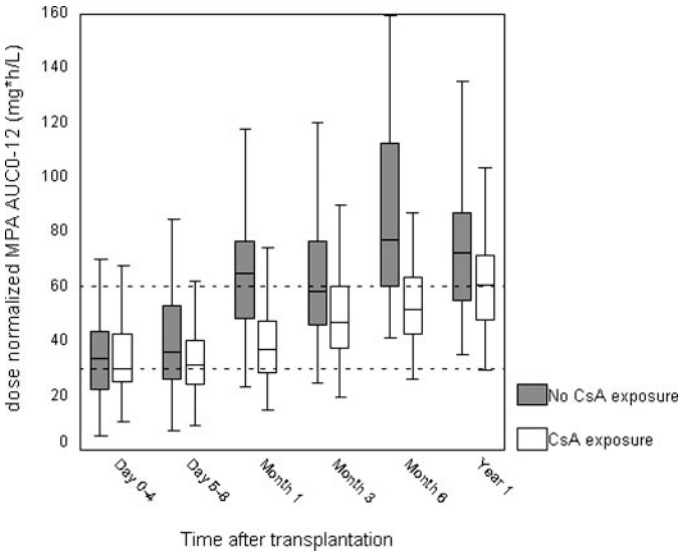

Figure 3. Course of dose-normalized MPA area-under-the-curve ($AUC_{0\ to\ 12}$) over time after transplantation for patients who had cyclosporine (CsA) as concomitant immunosuppressive therapy ($n = 144$ on days 0 to 4 posttransplantation; open box-whisker plots) and for patients who used an immunosuppressive regimen without CsA ($n = 102$ on days 0 to 4 posttransplantation; closed box-whisker plots). The box indicates the upper and lower quartiles, and the central line represents the median. The whiskers represent the 2.5 and the 97.5% values. The dotted lines represent the adopted therapeutic window for MPA $AUC_{0\ to\ 12}$ values of 30 to 60 mg/L per h (9). Exposure was significantly different between groups with $P < 0.05$ at months 1 and 6 and year 1. Exposure was significantly different between groups with $P < 0.01$ at month 3.

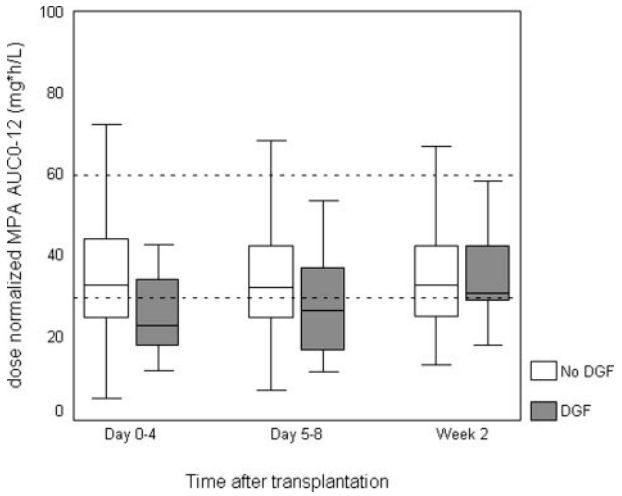

Figure 4. Course of dose-normalized MPA $AUC_{0\ to\ 12}$ over the first 2 wk after transplantation for patients with immediate graft function ($n = 212$ on days 0 to 4 posttransplantation; open box-whisker plots) and for patients with delayed graft function (DGF; $n = 34$ on days 0 to 4 posttransplantation; closed box-whisker plots). The box indicates the upper and lower quartiles, and the central line represents the median. The whiskers represent the 2.5 and the 97.5% values. The dotted lines represent the adopted therapeutic window for MPA $AUC_{0\ to\ 12}$ values of 30 to 60 mg/L per h (9). Exposure was significantly different between groups with $P < 0.05$ on days 0 to 4.

firms a slightly increased T_{max} in renal transplant recipients with diabetes. The increased T_{max} may be related to gastroparesis, which is present in many patients with diabetes (43) but does not have a clinically relevant impact on MPA exposure.

Figure 3 shows that dose-normalized MPA $AUC_{0\ to\ 12}$ increases over time after renal transplantation as a result of decreasing clearance. Given the identified relationships between MPA clearance and renal function, hemoglobin, albumin level, and cyclosporine predose level, this is in part caused by dynamic changes in these variables. The increase in exposure in the group without cyclosporine exposure occurs mainly in the first month after transplantation and thus may be caused by improving renal function, increasing albumin level, and climbing hemoglobin (Figure 3). Increasing MPA exposure later after transplantation may be the result, in part, of a decrease in cyclosporine predose levels (Table 2). This is illustrated in the cyclosporine group in Figure 3, in which median renal function and albumin level were stable between month 1 and year 1

(median renal function increased from 52 to 64 ml/min, and median albumin level increased from 37 to 39 g/L), whereas median cyclosporine predose level decreased from 237 to 155 ng/ml during that period. As a result of the gradual increase in MPA exposure, a subset of patients will be above the target window with standard MMF doses of 1000 mg twice daily after 6 to 12 mo after transplantation. This is most likely in patients who are no longer treated with cyclosporine and who have good renal function, albumin level, and hemoglobin (41). The increased MPA exposure may be very welcome in regimens in which cyclosporine is tapered or stopped, and in patients who tolerate such levels without toxicity, dose reductions may not be necessary. It is also important that the recommended target window (8) is based on a combination of MMF with a calcineurin inhibitor, and other target values may apply for other combinations (44).

Conclusion

With a population pharmacokinetic model, relationships have been identified between patient factors and pharmacokinetic parameters, thus explaining variability in MPA pharmacokinetics. Exposure to MPA is significantly influenced by renal function, albumin level, and hemoglobin and cyclosporine predose levels. These variables may prove to be useful for more effective therapeutic drug monitoring and MMF dosing, but this warrants further prospective research. Differences in MPA exposure between patients with or without DGF or between patients of different races are likely to be caused by the effect of renal function on MPA exposure. Diabetes and the use of gastric pH modulators other than antacids, corticosteroids, antibiotics, and antiviral agents do not have an effect on MPA exposure.

Acknowledgments

This study was supported by Roche Laboratories, Inc. M.D.P. received research support from Novartis, Roche, Wyeth, and Astellas; R.G. is an employee of Roche; R.D.M. works as an consultant for Roche; and T.V.G. has received research support from Novartis, Roche, and Wyeth.

We thank Sekhar Bhamidipati for creating the analytic data set and Dennis A. Hesselink for statistical assistance and inspiring discussions.

References

1. Halloran P, Mathew T, Tomlanovich S, Groth C, Hooftman L, Barker C: Mycophenolate mofetil in renal allograft recipients: A pooled efficacy analysis of three randomized, double-blind, clinical studies in prevention of rejection. *Transplantation* 63: 39–47, 1997
2. Kaufman DB, Shapiro R, Lucey MR, Cherikh WS, Bustami RT, Dyke DB: Immunosuppression: practice and trends. *Am J Transplant* 4[Suppl 9]: 38–53, 2004
3. Bullingham RES, Nicholls AJ, Kamm BR: Clinical pharmacokinetics of mycophenolate mofetil. *Clin Pharmacokinet* 34: 429–455, 1998
4. Shaw LM, Korecka M, Venkataramanan R, Goldberg L, Bloom R, Brayman KL: Mycophenolic acid pharmacodynamics and pharmacokinetics provide a basis for rational monitoring strategies. *Am J Transplant* 3: 534–542, 2003
5. Weber LT, Shipkova M, Armstrong VW, Wagner N, Schutz E, Mehls O, Zimmerhackl LB, Oellerich M, Tonshoff B: The pharmacokinetic-pharmacodynamic relationship for total and free mycophenolic acid in pediatric renal transplant recipients: A report from the German study group on mycophenolate mofetil therapy. *J Am Soc Nephrol* 13: 759–768, 2002
6. Van Gelder T, Hilbrands LB, Vanrenterghem Y, Weimar W, de Fijter JW, Squifflet JP, Hene RJ, Verpooten GA, Navarro MT, Hale MD, Nicholls AJ: A randomized double blind, multicenter plasma concentration study of the safety and efficacy of oral mycophenolate mofetil for the prevention of acute rejection after kidney transplantation. *Transplantation* 68: 261–266, 1999
7. Hale MD, Nicholls AJ, Bullingham RE, Hene R, Hoitsma A, Squifflet JP, Weimar W, Vanrenterghem Y, Van de Woude FJ, Verpooten GA: The pharmacokinetic-pharmacodynamic relationship for mycophenolate mofetil in renal transplantation. *Clin Pharmacol Ther* 64: 672–683, 1998
8. Shaw LM, Holt DW, Oellerich M, Meiser B, Van Gelder T: Current issues in therapeutic drug monitoring of mycophenolic acid: Report of a roundtable discussion. *Ther Drug Monit* 23: 305–315, 2001
9. Shaw LM, Korecka M, Aradhye S, Grossman R, Bayer L, Innes C, Cucciara A, Barker C, Naji A, Nicholls A, Brayman K: Mycophenolic acid area under the curve values in African American and Caucasian renal transplant patients are comparable. *J Clin Pharmacol* 40: 624–633, 2000
10. Hesselink DA, Van Hest RM, Mathot RA, Bonthuis F, Weimar W, de Bruin RW, van Gelder T: Cyclosporine interacts with mycophenolic acid by inhibiting the multidrug resistance-associated protein 2. *Am J Transplant* 5: 987–994, 2005
11. Van Hest RM, van Gelder T, Vulto AG, Mathot RA: Population pharmacokinetics of mycophenolic acid in renal transplant recipients. *Clin Pharmacokinet* 44: 1083–1096, 2005
12. Bennett WM: Immunosuppression with mycophenolate mofetil: One size does not fit all. *J Am Soc Nephrol* 14: 2414–2416, 2004
13. Johnson AG, Rigby RJ, Taylor PJ, Jones CE, Allen J, Franzen K, Falk MC, Nicol D: The kinetics of mycophenolic acid and its glucuronide metabolite in adult kidney transplant recipients. *Clin Pharmacol Ther* 66: 492–500, 1999
14. Borrows R, Chusney G, James A, Stichbury J, Van Tromp J, Cairns T, Griffith M, Hakim N, McLean A, Palmer A, Papalois V, Taube D: Determinants of mycophenolic acid levels after renal transplantation. *Ther Drug Monit* 27: 442–450, 2005
15. Kaplan B, Meier-Kriesche HU, Friedman G, Mulgaonkar S, Gruber S, Korecka M, Brayman KL, Shaw LM: The effect of renal insufficiency on mycophenolic acid protein binding. *J Clin Pharmacol* 39: 715–720, 1999
16. Kuypers DRJ, Vanrenterghem Y, Squifflet JP, Mourad M, Abramowicz D, Oellerich M, Armstrong V, Shipkova M, Daems J: Twelve-month evaluation of the clinical pharmacokinetics of total and free mycophenolic acid and its glucuronide metabolite in renal transplant recipients on low dose tacrolimus in combination with mycophenolate mofetil. *Ther Drug Monit* 25: 609–622, 2003
17. Johnson JH, Swan SK, Heim-Duthoy KL, Nicholls AJ, Tsina I, Tarnowski T: The pharmacokinetics of a single oral dose of mycophenolate mofetil in patients with varying degrees of renal function. *Clin Pharmacol Ther* 63: 512–518, 1998

18. Le Guellec C, Bourgoin H, Buchler M, Le Meur Y, Lebranchu Y, Marquet P, Paintaud G: Population pharmacokinetics and Bayesian estimation of mycophenolic acid concentrations in stable renal transplant patients. *Clin Pharmacokinet* 43: 253–266, 2004
19. Weber LT, Shipkova M, Lamersdorf T, Niedmann PD, Wiesel M, Mandelbaum A, Zimmerhackl LB, Schutz E, Mehls O, Oellerich M, Armstrong VW, Tonshoff B: Pharmacokinetics of mycophenolic acid (MPA) and determinants of MPA free fraction in pediatric and adult renal transplant recipients. *J Am Soc Nephrol* 9: 1511–1520, 1998
20. Kuypers DR, Claes K, Evenepoel P, Maes B, Coosemans W, Pirenne J, Vanrenterghem Y: Long-term changes in mycophenolic acid exposure in combination with tacrolimus and corticosteroids are dose dependent and not reflected by trough plasma concentration: A prospective study in 100 de novo renal allograft recipients. *J Clin Pharmacol* 43: 866–880, 2003
21. Zucker K, Rosen A, Tsaroucha A, De Faria L, Roth D, Ciancio G, Esquenazi V, Burke G, Tzakis A, Miller J: Unexpected augmentation of mycophenolic acid pharmacokinetics in renal transplant patients receiving tacrolimus and mycophenolate mofetil in combination therapy, and analogous in vitro findings. *Transpl Immunol* 5: 225–232, 1997
22. Cattaneo D, Perico N, Gaspari F, Gotti E, Remuzzi G: Glucocorticoids interfere with mycophenolate mofetil bioavailability in kidney transplantation. *Kidney Int* 62: 1060–1067, 2002
23. Pescovitz MD, Guasch A, Gaston R, Rajagopalan P, Tomlanovich S, Weinstein S, Bumgardner GL, Melton L, Sanwald Ducray P, Banken L, Hall J, Boutouyrie BX: Equivalent pharmacokinetics of mycophenolate mofetil in African-American and Caucasian male and female stable renal allograft recipients. *Am J Transplant* 3: 1581–1586, 2003
24. Van Hest RM, Mathot RA, Vulto AG, Le Meur Y, van Gelder T: Mycophenolic acid in diabetic renal transplant recipients: Pharmacokinetics and application of a limited sampling strategy. *Ther Drug Monit* 26: 620–625, 2004
25. Neylan J; for the US Renal Transplant Mycophenolate Mofetil Study Group: Immunosuppressive therapy in high-risk transplant patients: Dose-dependent efficacy of mycophenolate mofetil in African-American renal allograft recipients. *Transplantation* 64: 1277–1282, 1997
26. Sheiner LB: The population approach to pharmacokinetic data analysis: Rationale and standard data analysis methods. *Drug Metab Rev* 15: 153–171, 1984
27. Sheiner LB, Beal SL: Evaluation of methods for estimating population pharmacokinetic parameters. III. Monoexponential model: Routine clinical pharmacokinetic data. *J Pharmacokinet Biopharm* 11: 303–319, 1983
28. Sollinger HW: Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. US Renal Transplant Mycophenolate Mofetil Study Group. *Transplantation* 60: 225–232, 1995
29. The US Mycophenolate Mofetil Study Group: Mycophenolate mofetil for the prevention of acute rejection of primary cadaveric kidney transplants: Status of the MYC 1866 study at 1 year. *Transplant Proc* 29: 348–349, 1997
30. Ekberg H, Grinyo J, Nashan B, Vanrenterghem Y, Vincenti F, Calleja E, Nasmyth-Miller C, Truman M; on behalf of the CAEASAR Study Group: The use of daclizumab and mycophenolate mofetil in combination with corticosteroids and cyclosporine (low dose versus low dose followed by withdrawal) to optimize renal function in recipients of renal allografts [Abstract]. *Transplantation* 78: 458–459, 2004
31. Sheiner LB, Beal S, Rosenberg B, Marathe VV: Forecasting individual pharmacokinetics. *Clin Pharmacol Ther* 26: 294–305, 1979
32. Beal SL, Sheiner LB: *NONMEM User's Guides*, NONMEM Project Group, San Francisco, University of California at San Francisco, 1998
33. Karlsson MO, Sheiner LB: The importance of modeling interoccasion variability in population pharmacokinetic analyses. *J Pharmacokinet Biopharm* 21: 735–750, 1993
34. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16: 31–41, 1976
35. Poge U, Gerhardt T, Palmedo H, Klehr HU, Sauerbruch T, Woitas RP: MORD equations for estimation of GFR in renal transplant recipients. *Am J Transplant* 5: 1306–1311, 2005
36. Wahlby U, Thomson AH, Milligan PA, Karlsson MA: Models for time-varying covariates in population pharmacokinetic-pharmacodynamic analysis. *Br J Clin Pharmacol* 58: 367–377, 2004
37. Farrier DS: *PK Solutions 2.0, Noncompartmental Pharmacokinetics Data Analysis*, Summit Research Services, Montrose, CO 2000
38. Shaw LM, Mick R, Nowak I, Korecka M, Brayman KL: Pharmacokinetics of mycophenolic acid in renal transplant patients with delayed graft function. *J Clin Pharmacol* 38: 268–275, 1998
39. Benet LZ, Hoener B: Changes in plasma protein binding have little clinical relevance. *Clin Pharmacol Ther* 71: 115–121, 2002
40. Nowak I, Shaw LM: Mycophenolic acid binding to human serum albumin: Characterization and relation to pharmacodynamics. *Clin Chem* 41: 1011–1017, 1995
41. Buchler M, Lebranchu Y, Beneton M, Le Meur Y, Heng AE, Westeel PF, Le Guellec C, Libert F, Hary L, Marquet P, Paintaud G: Higher exposure to mycophenolic acid with sirolimus than with cyclosporine cotreatment. *Clin Pharmacol Ther* 78: 34–42, 2005
42. Kiberd BA, Lawen J, Fraser AD, Keough-Ryan T, Belitsky P: Early adequate mycophenolic acid exposure is associated with less rejection in kidney transplantation. *Am J Transplant* 4: 1079–1083, 2004
43. Talley NJ: Diabetic gastropathy and prokinetics. *Am J Gastroenterol* 98: 264–271, 2003
44. Van Gelder T, Shaw LM: The rationale for and limitations of therapeutic drug monitoring of mycophenolate mofetil in transplantation. *Transplantation* 8[Suppl]: S244–S253, 2005