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Limited Sampling Models and Bayesian Estimation for Mycophenolic Acid Area under the Curve Prediction in Stable Renal Transplant Patients Co-Medicated with Ciclosporin or Sirolimus

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Abstract

Background and Objective: Mycophenolate mofetil is a prodrug of mycophenolic acid (MPA), an immunosuppressive agent used in combination with corticosteroids and calcineurin inhibitors or sirolimus for the prevention of acute rejection after solid organ transplantation. Although MPA has a rather narrow therapeutic window and its pharmacokinetics show considerable intra- and interindividual variability, dosing guidelines recommend a standard dosage regimen of 0.5–1.0 g twice daily in adult renal, liver and cardiac transplant recipients. The main objective of the present study was to develop a method to predict the MPA area under the plasma concentration-time curve during one 12-hour dosing interval (AUC_{12}) by using multiple linear regression models and maximum *a posteriori* (MAP) Bayesian estimation methods in patients co-medicated with ciclosporin or sirolimus, aiming to individualize the dosage regimen of mycophenolate mofetil.

Patients and Methods: Pharmacokinetic profiles of MPA and mycophenolic acid glucuronide (MPAG), the main metabolite of MPA, were obtained from 40 stable adult renal allograft recipients on three different occasions: the day before switching from ciclosporin to sirolimus co-medication (±7.4 months post-transplantation; period I) and at 60 days and 270 days after the switch (periods II and III). Blood samples for determination of MPA and MPAG concentrations in plasma were taken at 0 hours (pre-dose) and at 0.33, 0.66, 1.25, 2, 4, 6, 8 and 12 hours after oral intake of mycophenolate mofetil. The MPA AUC_{12} was calculated by the trapezoidal method (the observed AUC_{12}). Patients were randomly divided into (i) a model-building test group (n = 27); and (ii) a model-validation group (n = 13). Multiple linear regression models were developed, based on three sampling times after drug administration. Subsequently, a population pharmacokinetic model describing MPA and MPAG plasma concentrations was developed using nonlinear mixed-effects modelling and a Bayesian estimator based on the population pharmacokinetic model was used to predict the MPA AUC_{12} based on three sampling times taken within 2 hours following dosing.

Results: Fifty-two percent of the observed AUC_{12} values (three periods) in the 40 patients receiving a fixed dose of mycophenolate mofetil 750 mg twice daily were outside the recommended therapeutic range (30–60 μg • h/mL). The failure of the standard dose to yield an AUC_{12} value within the therapeutic range was especially pronounced during the first study period. Of the multiple linear regression models that were tested, the equation based on the 0-hour (pre-dose), 0.66- and 2-hour sampling times showed the best predictive performance in the validation group: $r^2 = 0.79$, relative root mean square error (rRMSE) = 14% and mean relative prediction error (MRPE) = 0.9%. The pharmacokinetics of MPA and MPAG were best described by a two-compartment model with first-order absorption and elimination for MPA, plus a compartment for MPAG, also including a gastrointestinal compartment and enterohepatic cycling in the

case of sirolimus co-medication. The ratio of aminotransferase liver enzymes (AST and ALT) and the glomerular filtration rate significantly influenced MPA glucuronidation and MPAG renal excretion, respectively. Bayesian estimation of the MPA AUC_{12} based on 0-, 1.25- and 2-hour sampling times predicted the observed AUC_{12} values of the patients in the validation group, with the following predictive performance characteristics: $r^2 = 0.93$, rRMSE = 12.4% and MRPE = −0.4%.

Conclusion: Use of the developed multiple linear regression equation and Bayesian estimator, both based on only three blood sampling times within 2 hours following a dose of mycophenolate mofetil, allowed an accurate prediction of a patient's MPA AUC_{12} for therapeutic drug monitoring and dose individualization. These findings should be validated in a randomized prospective trial.

Background

Mycophenolate mofetil is an immunosuppressive agent used in combination with corticosteroids, calcineurin inhibitors or sirolimus for the prevention of acute rejection after solid organ transplantation.[1] Mycophenolate mofetil is a prodrug of mycophenolic acid (MPA), a reversible noncompetitive inhibitor of inosine monophosphate dehydrogenase, and blocks the *de novo* synthesis of guanosine nucleotide.[2] This not only results in the reduction of lymphocyte levels, the target effect, but also explains adverse effects such as diarrhoea, neutropenia and anaemia.[3,4]

When mycophenolate mofetil is orally administered to kidney transplant recipients, it undergoes de-esterification in the digestive tract and is converted into MPA, the active moiety, which is almost completely absorbed (bioavailability [F] = 0.97). MPA is extensively bound to albumin (97–99%)[5] and is metabolized in the liver and the intestinal mucosa, mainly to a phenolic glucuronide (MPAG) and, to a lesser extent, to mycophenolate acyl glucuronide, which has been shown to have *in vitro* immunosuppressive activity. Other minor metabolites identified in humans include the 7-*O*-glucoside of MPA and 6-*O*-desmethyl-MPA.[5] MPAG, the major and inactive metabolite, is mainly eliminated by renal excretion and has been shown to undergo enterohepatic cycling, particularly in studies conducted during the early post-transplantation period.[6]

Current manufacturer guidelines recommend a standard dose of mycophenolate mofetil for all patients within a transplant group, e.g. 0.5–1.0 g given twice daily in adult renal, liver and cardiac transplant recipients.[7,8] The pharmacokinetics of MPA, however, are characterized by considerable intra- and inter-patient variability.[9-11] In addition, MPA has a narrow therapeutic window. As a consequence, dose individualization and MPA therapeutic drug monitoring to determine the actual exposure may improve the efficacy and tolerability of mycophenolate mofetil. Mourad et al.[12] have demonstrated a significant relationship between the MPA trough or pre-dose MPA plasma concentration (C_0) or the area under the MPA plasma concentration-time curve during one 12-hour dosing interval (AUC_{12}) and the risk of rejection and haematological adverse effects. However, a stronger pharmacokinetic-pharmacodynamic relationship between acute rejection and the AUC_{12} compared with the C_0 favours AUC_{12}-based rather than C_0-based therapeutic drug monitoring. Targeting an MPA AUC_{12} of 30–60 μg • h/mL has been proposed to minimize the risk of acute rejection and to reduce toxicity.[6]

To estimate an individual patient's AUC_{12} without measuring the full MPA plasma concentration-time profile, two different methods can be used. A limited sampling strategy (LSS) based on multiple linear regression (MLR) models using a small number of blood samples, preferably obtained in the early post-dose period, can be used to predict the full AUC_{12}. This approach, however, can be inconvenient in that it requires strict adherence to the set times for blood sample collection which, in practice, may not be easy. Maximum *a priori* (MAP) Bayesian estimation of the AUC_{12} for each individual patient is also based on a limited number of plasma concentration measurements, preferably in the early post-dose period, but involves more complex calculations and requires the development of a population pharmacokinetic model. Unlike the LSS, however, which requires strict adherence to the time of blood sample collection, the MAP Bayesian procedure is flexible in blood sample timing.

The main objective of the present study was to develop a method to predict the MPA AUC_{12} by using MLR models and MAP Bayesian estimation methods in patients co-medicated with ciclosporin or sirolimus, aiming to individualize the dosage regimen of mycophenolate mofetil. Secondary objectives were to characterize MPA and MPAG pharmacokinetics in 40 stable renal transplant patients who were successively co-medicated with ciclosporin or sirolimus between approximately 7 and 16 months post-transplantation; to identify and model the effect of demographic and clinical factors on pharmacokinetic variability by using nonlinear mixed-effect modelling techniques and to assess the need for MPA

therapeutic drug monitoring during the 7- to 16-month post-transplantation period based on AUC_{12} values at different periods post-transplantation.

Patients and Methods

Patient Characteristics and Study Design

Data from 40 stable adult renal allograft recipients, transplanted in one of two Belgian university hospitals (Universitair Ziekenhuis Brussel and Universitair Ziekenhuis Antwerpen) were included in this study. The study was approved by the local ethics committees and all patients signed an informed consent form. All patients received mycophenolate mofetil (1 g twice daily), ciclosporin and corticosteroids, all orally, during the initial post-transplantation period. One month prior to the switch from ciclosporin to sirolimus, the dose of mycophenolate mofetil was reduced to 0.75 g twice daily. At 7.4 ± 1.4 months, ciclosporin was replaced by sirolimus while continuing mycophenolate mofetil (0.75 g twice daily) and corticosteroid treatment. The safety and efficacy aspects related to the switch from ciclosporin to sirolimus as co-medication have been published before.[13] Full pharmacokinetic profiles for MPA and MPAG during one dosing interval were determined on three different occasions: (i) on the day before switching from ciclosporin to sirolimus at 7.4 ± 1.4 months (n = 40, period I); (ii) at 60 days after the switch (n = 39, period II); and (iii) at 270 days after the switch (n = 36, period III). For the determination of the full pharmacokinetic profiles, blood samples were collected in EDTA tubes and the plasma was kept frozen at –20°C until analysis. Sampling times were as follows: at 0 hours (pre-dose) and at 0.33, 0.66, 1.25, 2, 4, 6, 8 and 12 hours following mycophenolate mofetil administration.

Analytical Method

MPA and MPAG plasma concentrations were determined by a validated high-performance liquid chromatography (HPLC) method with UV diode array detection. Calibrators prepared in drug-free plasma were used and their concentrations were 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 15.0 and 20.0 μg/mL for MPA and 2, 5, 10, 20, 50, 100, 150 and 200 μg/mL for MPAG. In addition, in-house quality control (QC) samples at three different concentrations (0.75, 2 and 10 μg/mL for MPA and 7.5, 20.0 and 100.0 μg/mL for MPAG) were used. Using 1.5 mL polystyrene tubes, 20 μL of an internal standard solution (50 μg visnadine/mL acetonitrile) was added to a 500 μL aliquot of each plasma calibrator or of a patient's plasma sample. The plasma samples were subsequently deproteinized by successively adding 20 μL of 5% perchloric acid and 20 μL of 50% sodium tungstate followed by vortex mixing for 30 seconds. Plasma concentrations of MPA and MPAG were measured by a reverse-phase HPLC method using a Supersher C18© column (3.9 × 300 mm; Waters Corporation, Milford, MA, USA) at 75°C. The mobile phase consisted of a gradient triethyl ammonium phosphate (TEAP) buffer/acetonitrile (85/15 up to 30/70 v/v) and was pumped through the column at a flow rate of 1 mL/min. The TEAP buffer was adjusted to pH 3.0 with phosphoric acid. MPA and MPAG were detected by UV absorbance at a wavelength of 254 nm. The average extraction recoveries of MPA and MPAG were 95–99%. The calibration curves were linear: $r^2 \geq 0.99$ for both MPA and MPAG. The intra- and interday imprecision values for both analytes were less than 5% and 10%, respectively, for all tested in-house QCs. This method was found to be precise and accurate on an intra- and interday basis: mean relative prediction error (MRPE) <11.3% for all QC samples tested.

Noncompartmental Pharmacokinetic Analysis

The AUC_{12} was estimated by using the trapezoidal method (noncompartmental analysis, WinNonlin® version 5.01; Pharsight Corporation, Mountain View, CA, USA). Pharmacokinetic parameters of MPA and MPAG, such as the elimination half-life, apparent oral clearance (CL/F) and apparent volume of distribution (V_d), were calculated by using standard equations. Other parameters calculated were the maximum MPA concentration and the time to reach the maximum MPA concentration.

Multiple Linear Regression and Limited Sampling Strategies

The sample (n = 40) was randomly split into two groups: (i) a model-building subgroup (the test group) comprising 27 patients; and (ii) a model-validation subgroup of the remaining 13 patients. Limited sampling strategies were developed to predict MPA AUC_{12} values calculated on the basis of the full pharmacokinetic profiles (nine MPA plasma concentrations determined during one dosing interval) by MLR (JMP 6®; SAS Institute Inc., Cary, NC, USA) using various combinations of two to three MPA plasma concentrations determined during the 2-hour interval following mycophenolate mofetil dosing in the test group. The predicted AUC_{12} from each model was compared with the observed AUC_{12} in the validation subgroup. The predictive performance of the best model was further internally evaluated in the model-building group by repeated

cross-validation as described by Pawinski et al.[14] Briefly, the dataset was repeatedly and randomly divided into two equal groups: a training group and an evaluation group. This process was repeated a total of 20 times. The training group records were used to determine the relationship (i.e. regression coefficients) between the observed MPA AUC_{12} and the sampling times retained in the best MLR model. The linear regression equations obtained in the preceding step were used to estimate the MPA AUC_{12} for the profiles in the corresponding evaluation set. Residuals were calculated for each of the MPA AUC_{12} values in the evaluation group by taking the difference between the logarithm of the reference MPA AUC_{12} and the logarithm of the MPA AUC_{12} estimated by the regression equation. The distribution of the entire set of residuals was examined to ensure that the selected limited sampling equation for the prediction of the MPA AUC_{12} generated a distribution of estimated MPA AUC_{12} values in the evaluation sets that met certain statistical criteria (mean value for the entire set of residuals close to 0 and with a coefficient of variation [CV] <30%).

Population Pharmacokinetic Analysis

Nonlinear mixed-effects modelling was performed by using NONMEM® version VI (double precision; Icon Development Solutions, LLC, Ellicott City, MD, USA) and PsN-toolkit,[15] a programming library containing a collection of computer intensive statistical methods for nonlinear mixed-effects modelling and Xpose 4.0,[16] an S-Plus-based population pharmacokinetic-pharmacodynamic model-building aid for NONMEM® with an interface containing graphical and statistical tools. The same two groups generated for stepwise MLR were used: (i) a model-building subgroup (test group) comprising 27 patients; and (ii) a model-validation subgroup of the remaining 13 patients, used for Bayesian estimation. The first-order conditional estimation approach with interaction between parameters (FOCEI) was used throughout the entire modelling process. As shown in equations 1 and 2, allometric scaling was used throughout the modelling process: bodyweight (WT) was linearly incorporated into clearance (CL), intercompartmental clearance (Q) and V_d terms, with exponents fixed to 0.75 for CL and Q and to 1 for V_d terms.[17] CL and V_d for a typical subject with a bodyweight of WT_i were predicted from the structural model as follows:

$$TVV_{d(i)} = \theta_{v_d} \times \frac{WT_i}{WT_{Med}} \quad \text{(Eq. 1)}$$

$$TVCL_i = \theta_{CL} \times \left(\frac{WT_i}{WT_{Med}}\right)^{0.75} \quad \text{(Eq. 2)}$$

where $TVV_{d(i)}$ and $TVCL_i$ are the typical values of V_d and CL estimated by NONMEM® with allometric scaling, θ_{v_d} and θ_{CL} are the respective values estimated without allometric scaling, and WT_{Med} is the median bodyweight of the sample set.

The structural pharmacokinetic model was built in two steps:

(i) First, MPA and MPAG plasma concentrations (after conversion to molar MPA equivalents) were modelled separately and various structural models were tested: one-, two- and three-compartment models with first-order or zero-order absorption and with or without a lag time (t_{lag}). MPA concentrations were modelled alone for 'parsimony' reasons, whereas MPAG concentrations were separately modelled solely to facilitate further building of the combined model.

(ii) Subsequently, MPA and MPAG concentrations (MPA molar equivalents) were simultaneously used to build the structural pharmacokinetic model. The influence of enterohepatic cycling was tested during this second stage. For 'parsimony' reasons, simpler models were also tried on the combined MPA and MPAG plasma concentration-time data. Basic pharmacokinetic parameters of MPA and MPAG were estimated by NONMEM® in terms of first-order rate constants (k) and apparent volumes of distribution of the various compartments. CL and Q were further computed using conventional equations. For example, when a two-compartment model was tested, equations 3 and 4 were used to estimate CL and Q from the first-order rate constants:

$$CL = k_e \times V_2 \quad \text{(Eq. 3)}$$

$$Q = k_{23} \times V_2 = k_{32} \times V_3 \quad \text{(Eq. 4)}$$

where k_e is the first-order elimination rate constant, V_2 is the central compartment volume, V_3 is the peripheral compartment volume, k_{23} and k_{32} are the first-order transfer rate constants from the central to the peripheral compartment and from the peripheral to the central compartment, respectively. Since oral bioavailability (F) could not be determined, the values for MPA CL, V_d and Q correspond to the ratios CL/F, V_d/F and Q/F. In the absence of urine data, the fraction of the MPA dose converted to MPAG (f_m) is not precisely known. Therefore, the values for MPAG V_d and CL correspond to the ratios V_d/f_m and CL/f_m.

Intra- and interindividual variabilities were modelled using an exponential error model and initially all parameters were tested.

The value of a parameter in the i^{th} individual at the j^{th} occasion (P_{ij}) was a function of the parameter value in the typical individual (θ_i) and an individual deviation initially represented by η_i and κ_{ij}, the interindividual and the intra-individual variability terms for the j^{th} occasion for the i^{th} patient. The ηs

and κs in the population were supposed to be symmetrically distributed, zero-mean random variables with a variance that was estimated as part of the model estimation from equation 5:

$$P_{ij} = \theta_i \times \exp(\eta_i + \kappa_{ij}) \quad \text{(Eq. 5)}$$

η and/or κ terms were maintained in the structural model only when they improved the model based on the decrease of the Akaike information criterion (AIC) computed as described below.

Additive, proportional, exponential and mixed error models were tested for the residual error from equations 6–9:

$$Y = IPRED + \varepsilon_{addX} \quad \text{(Eq. 6)}$$

$$Y = IPRED \times (1 + \varepsilon_{propX}) \quad \text{(Eq. 7)}$$

$$Y = IPRED \times (1 + \exp(\varepsilon_{expX})) \quad \text{(Eq. 8)}$$

$$Y = IPRED \times (1 + \varepsilon_{propX}) + \varepsilon_{addX} \quad \text{(Eq. 9)}$$

where Y represents the observed concentration, IPRED is the individual predicted concentration and ε_{addX}, ε_{propX} and ε_{expX} are the additive, the proportional and the exponential error terms on the substance 'X' concentrations, respectively. εs are symmetrically distributed, zero-mean random variables with variances that are estimated as part of the population model-fitting process from equations 6–9.

Model selection only concerned models for which the NONMEM® minimization process was successful and was based on the following criteria: the AIC, the plausibility and the precision of parameter estimates and graphical analysis. The AIC was computed on the model objective function value (OFV) and the number of parameters used (NPAR) as follows:

$$AIC = OFV + (2 \times NPAR) \quad \text{(Eq. 10)}$$

The models with the lowest AIC were further evaluated. The precision of parameter estimates, expressed as the standard error of estimates, was generated by the co-variance option within the NONMEM® program. Goodness-of-fit plots including individual predictions versus observed concentrations, as well as conditional weighted residuals (CWRES)[18] versus predictions, and the distribution of CWRES with time after dose, were used for diagnostic purposes.

To explain interpatient, interoccasion, and residual variability on pharmacokinetic parameters, relationships were investigated between pharmacokinetic parameters and the following patient co-variates: age, sex, race, bodyweight, glomerular filtration rate (GFR) estimated by the Cockcroft and Gault and Nankivell formulas,[19,20] plasma albumin concentration, liver enzymes (AST and ALT), serum bilirubin concentration and haemoglobin. The use of either sirolimus or ciclosporin as co-medication was also tested. Individual Bayesian estimates of pharmacokinetic parameters were generated from the structural model and a stepwise regression model was built between each co-variate and the individual pharmacokinetic parameters using NONMEM®. A difference of at least 3.84 in the OFV (χ^2 p-value ≤0.05) from the structural model OFV was considered statistically significant.

Co-variates that were continuous variables (age, bodyweight, GFR, plasma albumin concentration, AST, ALT, serum bilirubin concentration and haemoglobin) were centred to their median values and tested on the pharmacokinetic parameters in a linear (equation 11) or nonlinear (equation 12) manner. For example:

$$k_{40} = \theta_{k_{40}} + \theta_{GFR\ on\ k_{40}} \times \frac{GFR_i}{GFR_{Med}} \quad \text{(Eq. 11)}$$

$$k_{40} = \theta_{k_{40}} + \left(\frac{GFR_i}{GFR_{Med}}\right)^{\theta_{GFR\ on\ k_{40}}} \quad \text{(Eq. 12)}$$

where k_{40} is the first-order elimination rate constant from compartment 4, GFR_i is the GFR estimated by the Nankivell formula for the i^{th} individual and GFR_{Med} represents the median GFR estimated by the Nankivell formula.[20]

For the categorical co-variates such as sex, race, time after the transplantation (occasions 1, 2 and 3 represented 6, 9 and 16 months post-transplantation, respectively) and the use of sirolimus or ciclosporin as co-medication, a change in a pharmacokinetic parameter, e.g. k_{41}, was evaluated by equation 13:

$$k_{41} = \begin{cases} \theta_{k_{41}} \text{ in the case of ciclosporin co-medication} \\ \theta_{k_{41}} + \theta_{sir\ on\ k_{41}} \text{ in the case of sirolimus co-medication} \end{cases} \quad \text{(Eq. 13)}$$

where $\theta_{k_{41}}$ is the population average first-order transfer rate constant from compartment 4 to compartment 1 (k_{41}) for patients co-medicated with ciclosporin and $\theta_{sir\ on\ k_{41}}$ is the fractional change in k_{41} for patients co-medicated with sirolimus.

Certain continuous co-variates were also categorized and tested as described above and/or combined and tested as a unique factor. For example, GOT and GPT were combined and categorized as follows (equation 14):

$$CL = \begin{cases} \theta_{CL} \times \left(\frac{WT}{WT_{Med}}\right) \text{ in the case of } \frac{AST}{ALT} \leq 1 \\ \theta_{CL} \times \left(\frac{WT}{WT_{Med}}\right) + \theta_{AST/ALT\ on\ CL} \text{ in the case of } \frac{AST}{ALT} > 1 \end{cases} \quad \text{(Eq. 14)}$$

where θ_{CL} is the population average CL for patients with an AST/ALT ratio ≤1 and $\theta_{AST/ALT\ on\ CL}$ is the fractional change in CL for patients with an AST/ALT ratio >1.

Subsequently, a full model was built using NONMEM® including all co-variates that showed significant influence on pharmacokinetic parameters. A backward process was implemented to build the final model. To partially compensate for the multiple comparisons, a more restrictive criterion was adopted and a difference in the OFVs of >11 (χ^2 p-value ≤0.001) was required to maintain the co-variate in the final model. Bootstrapping, cross-validation and simulations were used to validate the final model. Two-hundred bootstraps were generated using the PsN toolkit and a confidence interval was built around the median of each parameter. Estimated values of each parameter by the final model were compared with this confidence interval. During the cross-validation, 'predicted' estimates of the OFV were obtained with the final model by successively removing one different subject from the dataset. The model with estimates acquired for subset N-1 was applied to the remaining subject. The root mean square error (RMSE) and MRPE were computed on the OFVs. Values higher than 30% would suggest the presence of influential individuals. Finally, the predictive performance of the model was evaluated using a visual predictive check (VPC). The population pharmacokinetic model was used to simulate 1000 hypothetical patients. The distribution (median and 5th and 95th percentiles) of the simulated concentration-time curves was compared with the observed MPA and MPAG concentration values in the original dataset.

Bayesian Estimation

Bayesian estimation on the validation group (by the POSTHOC and MAXEVAL = 0 option of the NONMEM® estimation subroutine) was performed by using the final model developed on the model-building patient group. All combinations of three MPA plasma concentration-time points sampled within 2 hours following mycophenolate mofetil dosing were tested and the best combination was retained. Previously published Bayesian estimators developed for adult stable renal transplant recipients were also assessed in patients allocated to the validation group.[21,22] Observed AUC_{12} values (obtained by the trapezoidal rule) were compared with AUC_{12} values computed using Bayesian estimators as described in the following section.

Evaluation of Predictive Performance of Predictors of the Area under the Plasma Concentration-Time Curve from 0 to 12 Hours

Linear regression was performed to evaluate the strength of the relationship between the AUC_{12} values predicted by the various LSS/Bayesian estimators and the observed AUC_{12} values. The Pearson coefficient of determination (r^2) was one of the criteria used to select the best LSS and Bayesian estimator. In addition, the predictive performance of the various LSSs and agreement between predicted and observed AUC_{12} values were assessed as described by Sheiner and Beal[23] and Bland and Altman,[24] respectively. Sheiner and Beal[23] described two parameters: the RMSE to characterize the precision of the model and the prediction error (PE) to estimate the bias on each difference between the predicted and observed AUC_{12}. The lower the RMSE and PE values, the better the model. Bland and Altman[24] used the 95% confidence interval around the MRPE to assess the predictive performance of the LSS. Equations 15–17 display expressions of estimation of the relative root mean squared error (rRMSE), relative PE (RPE) and MRPE, respectively. Finally, during the evaluation of predictive performance in this study, a model was considered to display a good predictive performance when, in the validation sample set, the 95% confidence interval around the MRPE was included between −20% and +20%[20] of the reference MPA AUC_{12} values.

$$\text{rRMSE} = \frac{1}{N}\sqrt{\sum\left(\frac{AUC_{pred} - AUC_{obs}}{AUC_{obs}} \times 100\right)^2} \quad \text{(Eq. 15)}$$

$$\text{RPE} = \left(\frac{AUC_{obs} - AUC_{pred}}{AUC_{obs}}\right) \times 100 \quad \text{(Eq. 16)}$$

$$\text{MRPE} = \frac{1}{N}\sum\left(\frac{AUC_{obs} - AUC_{pred}}{AUC_{obs}} \times 100\right) \quad \text{(Eq. 17)}$$

where AUC_{obs} represents the observed AUC_{12} and AUC_{pred} represents the AUC_{12} predicted by the model.

Therapeutic Drug Monitoring of Mycophenolic Acid

The need for therapeutic drug monitoring to optimize mycophenolate mofetil dosage was assessed based on an MPA therapeutic window of 30–60 μg • h/mL for the AUC_{12}. AUC_{12} values obtained in the complete patient dataset (dose: 0.75 g twice daily) were computed for the three different periods following transplantation. The percentage of AUC_{12} values outside the therapeutic range, thus obtained for the three different periods post-transplant, was then calculated.

Table I. Patient characteristics in the test and validation groups

Patient characteristics	Test group[a]	Validation group[a]
No. of patients	27[b]	13[c]
Sex (n; male/female)	18/9	8/5
Age (y)	54 [24–65]	47 [32–67]
Bodyweight (kg)	70 [58–101]	67 [43–132]
MMF daily dose (g)	1.5 [1.5–1.5]	1.5 [1.5–1.5]
Prednisolone daily dose (mg)	7.5 [5–10]	7.5 [5–12.5]
Ciclosporin C_0 (ng/mL)	174 [103–207]	166 [127–215]
Sirolimus C_0 (ng/mL)	10.5 [6–13.4]	11.5 [7.3–17.2]
Serum ALT (U/L)	28 [19–46]	20 [13–42]
Serum AST (U/L)	34 [15–44]	42 [19–62]
GFR[d] (mL/min)	55 [45–69]	61 [53–81]

a Values are expressed as median [range] unless specified otherwise.

b n = 27 in period I and II, and n = 24 in period III (see Patient Characteristics and Study Design section).

c n = 13 in period I, and n = 12 in periods II and III (see Patient Characteristics and Study Design section).

d Calculated GFR (by the Nankivell formula).[20]

C_0 = trough plasma concentration; **GFR** = glomerular filtration rate; **MMF** = mycophenolate mofetil.

Results

Clinical characteristics of the patients in the test and validation groups are summarized in table I. There were no significant differences between the validation and test group characteristics. Figure 1 shows individual MPA and MPAG plasma concentration-time profiles. Large pharmacokinetic variability was observed during the three study periods. Pharmacokinetic and exposure parameters of MPA calculated by noncompartmental analysis are summarized in table II. They are characterized, in almost all cases, by a high interindividual variability. Indeed, in all but three cases, the CV of the pharmacokinetic parameters was >30%. No statistically significant differences were found in the pharmacokinetic parameters between the test and the validation groups.

Figure 2 shows box-and-whisker plots of observed AUC_{12} values in the studied patients, who received a fixed dose regimen of mycophenolate mofetil 1.5 g/day, for the three different periods following transplantation. Fifty-two percent of the AUC_{12} values of 40 patients were outside the therapeutic range for the AUC_{12}, i.e. 30–60 μg • h/mL. Especially during the first observation period, when the patients were co-medicated with ciclosporin, 63% of the AUC_{12} values were outside the

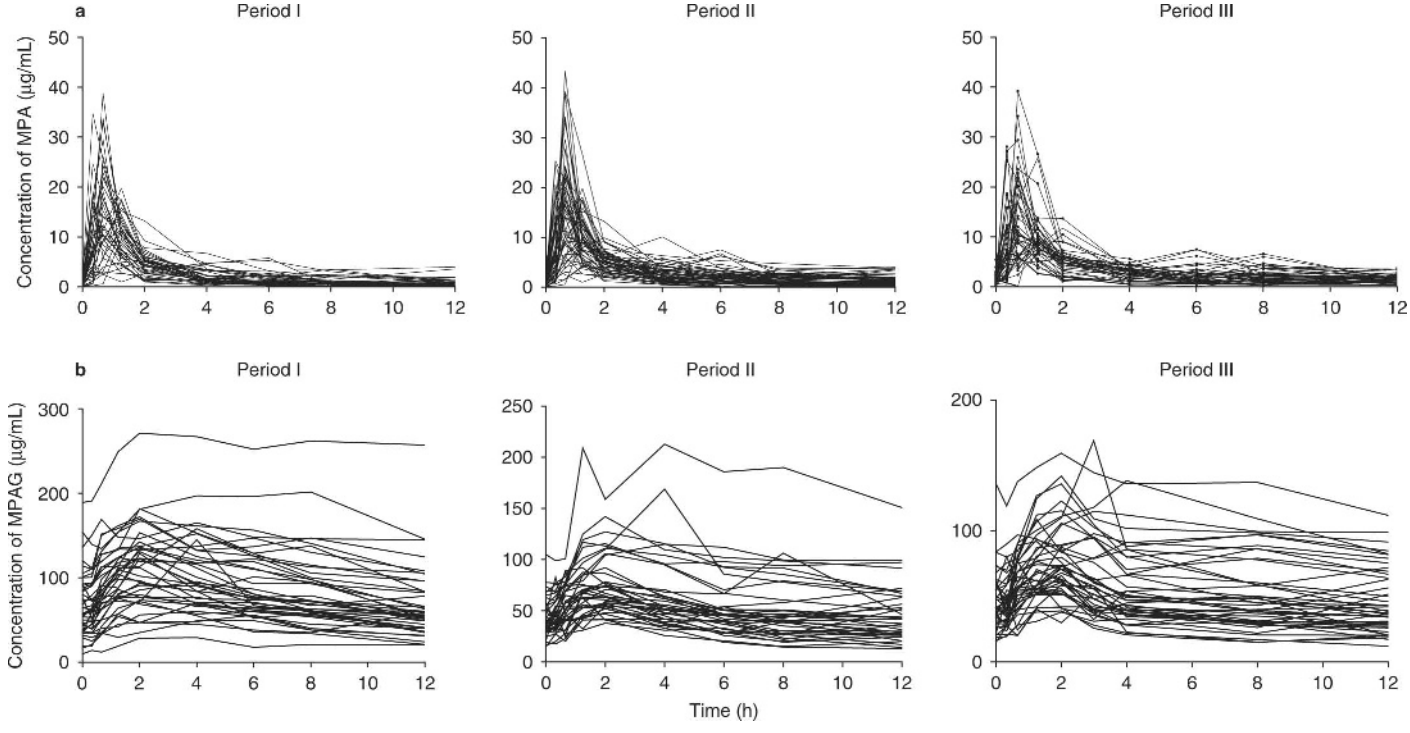

Fig. 1. (**a**) Mycophenolic acid (MPA) and (**b**) mycophenolic acid glucuronide (MPAG) individual pharmacokinetic profiles during period I (± 7 months), period II (± 9 months) and period III (± 15 months) [n = 40, 39 and 36, respectively].

Table II. Pharmacokinetic and exposure parameters of mycophenolic acid obtained by noncompartmental analysis

Parameter	Test group[a]	Validation group[a]	p-Value[b]
AUC_{12} (μg • h/mL)			
Period I	37.1 [23–65]	38.3 [20–49]	0.81
Period II	42.2 [19–81]	52 [18–94]	0.51
Period III	54.1 [14–79]	56.2 [14–71]	0.95
C_{min} (μg/mL)			
Period I	1.1 [0.2–2.4]	0.8 [0.1–2.9]	0.67
Period II	1.6 [0.1–6]	1.7 [0.2–5]	0.41
Period III	1.5 [0.2–10]	1.5 [0.2–7]	0.59
C_{max} (μg/mL)			
Period I	16.5 [9–39.2]	16.1 [8–40]	0.67
Period II	18.6 [4.2–43.3]	16.3 [0.3–41.2]	0.53
Period III	12.8 [7–39.]	15.1 [6.2–24]	0.18
t_{max} (h)			
Period I	1 [0.3–1.3]	1 [0.3–1.3]	0.79
Period II	1 [0.3–2]	0.66 [0.3–2]	0.48
Period III	1 [0.3–2]	1 [0.3–2]	0.51
CL/F (L/h)			
Period I	12.3 [7.6–21]	15.6 [6–35]	0.43
Period II	9.5 [3–29]	15 [2–42]	0.23
Period III	16.2 [7–92]	15 [3–53]	0.73
V_d/F (L)			
Period I	61 [28–123]	64.3 [27–184]	0.62
Period II	63 [24–126]	61.8 [17–109]	0.67
Period III	78 [20–98]	70 [33–142]	0.49
$t_{½}$ (h)			
Period I	4 [1.3–6]	7 [2–12.1]	0.54
Period II	4 [1.3–13]	5 [4–12.4]	0.11
Period III	5 [2–8.3]	6 [3–12]	0.96

a Values are expressed as median [range].

b p-Value of the Wilcoxon rank test.

AUC_{12} = area under the plasma concentration-time curve during one 12-hour dosing interval; **CL/F** = apparent oral clearance; **C_{max}** = maximum plasma concentration; **C_{min}** = minimum plasma concentration; **$t_{½}$** = elimination half-life; **t_{max}** = time to reach C_{max}; **V_d/F** = apparent volume of distribution after oral administration.

therapeutic range. Although the median AUC_{12} values during periods 2 and 3 were located within the therapeutic range, 47% of patients had AUC_{12} values outside the 30–60 μg • h/mL range.

Stepwise MLR analysis was used to select MPA plasma concentration sampling timepoints within the 0–2 hour post-dose interval. Model equations are shown in table III together with measures of correlation (Pearson r^2), accuracy (MRPE) and precision (rRMSE). Model 1, with samples drawn at 0 hours (pre-dose), 0.66 hours (40 minutes) and 2 hours after mycophenolate mofetil dosing, showed not only the best fit to the MPA AUC_{12} ($r^2 = 0.79$), but also better prediction precision and accuracy than the other models. In addition, with this model, none of the patients had a predicted AUC_{12} lower than –20% or higher than +20% of the reference value. This model was additionally validated by 20 repeated cross-validations and gave good performance (mean value for the entire set of residuals = –0.03 and CV = 23%).

As far as the basic deterministic pharmacokinetic model for the population analysis is concerned, a two-compartment model with t_{lag} and first-order absorption and elimination best fitted the MPA plasma concentrations, whereas a one-compartment model with t_{lag} and first-order absorption and elimination was retained for fitting the MPAG plasma concentrations. A two-compartment model with t_{lag}, first-order absorption, intercompartmental transfer and elimination rate constants plus a gastrointestinal tract (GIT) and an MPAG compartment best fitted the combined MPA and MPAG data. A schematic diagram of the latter model is shown in figure 3. For MPA, the following pharmacokinetic parameters were estimated: t_{lag}, absorption rate constant (k_a), apparent volume of the central compartment after

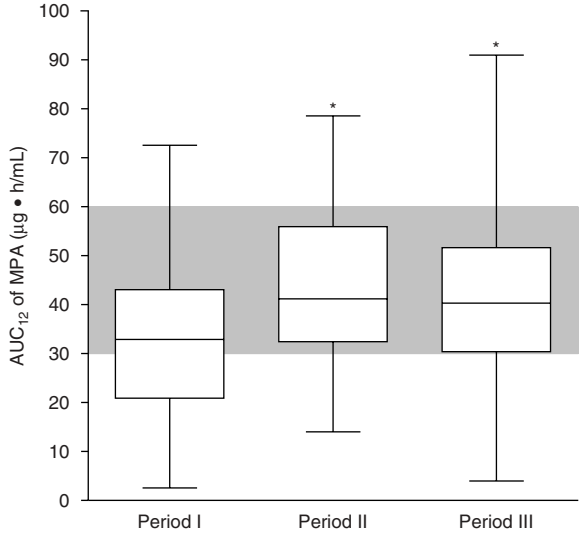

Fig. 2. Box and whisker plots of observed area under the plasma concentration-time curve during one 12-hour dosing interval (AUC_{12}) values in the studied patient population receiving a fixed dosage regimen of mycophenolate mofetil 1.5 g/day at three different post-transplantation times: period I (±7 months), period II (±9 months) and period III (±15 months). The boxes represent the 25th, 50th and 75th percentiles, the whiskers represent the range, the grey shaded area represents the therapeutic range for the AUC_{12} (i.e. 30–60 μg • h/mL) and the asterisks represent statistically significant differences from the period I AUC_{12} (Kruskal-Wallis [paired] test on the differences). **MPA** = mycophenolic acid.

Table III. Multiple linear regression models: predicted mycophenolic acid area under the plasma concentration-time curve during one 12-hour dosing interval (AUC_{12}) correlated with the observed AUC_{12}

Model	Sampling times (h)	Model equation	r^2	rRMSE (%)	MRPE (%) [95% CI]
1	0, 0.66, 2	$8.64 + 5.13 \bullet C_0 + 0.62 \bullet C_{0.66} + 2.84 \bullet C_2$	0.79	14	0.9 [–2.7, 1.6]
2	0, 0.33, 2	$10.69 + 4.90 \bullet C_0 + 0.58 \bullet C_{0.33} + 3.33 \bullet C_2$	0.73	26	1.6 [–0.5, 7.6]
3	0, 1.25, 2	$10.09 + 6.39 \bullet C_0 + 1.03 \bullet C_{1.25} + 1.96 \bullet C_2$	0.73	27	1.9 [1.5, 7]
4	0, 0.66, 1.25	$10.29 + 5.17 \bullet C_0 + 0.44 \bullet C_{0.66} + 1.26 \bullet C_{1.25}$	0.70	33	2.2 [–5.4, 2.4]
5	0, 0.33, 1.25	$8.35 + 7.04 \bullet C_0 + 0.54 \bullet C_{0.33} + 1.71 \bullet C_{1.25}$	0.69	35	2.2 [2, 7.5]
6	0.33, 0.66, 2	$7.86 + 0.56 \bullet C_{0\ .33} + 0.58 \bullet C_{0.66} + 3.95 \bullet C_2$	0.67	36	–3.2 [–8.5, –0.3)]
7	0.33, 1.25, 2	$7.29 + 0.89 \bullet C_{0.33} + 0.90 \bullet C_{1.25} + 3.50 \bullet C_2$	0.62	41	–3.9 [–8.4, –0.2]
8	0.66, 1.25, 2	$10.95 + 0.703 \bullet C_{0.66} + 0.17 \bullet C_{1.25} + 3.5 \bullet C_2$	0.62	41	–4 [–9.4, –0.3]
9	0, 2	$15.53 + 5.84 \bullet C_0 + 2.98 \bullet C_2$	0.67	47	2.7 [2, 8.6]
10	0, 1.25	$12.8 + 7.70 \bullet C_0 + 1.57 \bullet C_{1.25}$	0.64	48	5.41 [3.2, 11.1]

C_x = plasma concentration at *x* hour(s); **MRPE** = mean relative prediction error; **rRMSE** = relative root mean square error.

oral administration (V_2/F), apparent volume of the peripheral compartment after oral administration (V_3/F), intercompartmental clearance ($Q/F = k_{23} \bullet V_2/F = k_{32} \bullet V_3/F$), first-order rate constants for elimination from the central compartment (k_{20}) and biotransformation (glucuronidation) to MPAG apparent clearance ($CL/F = k_{24} \bullet V_2/F$). For MPAG, the apparent volume of distribution (V_4/f_m) and first-order rate constants for renal elimination (k_{40}) and enterohepatic cycling (k_{41}) were estimated (table IV).

The GFR estimated by the Nankivell formula was the only covariate maintained in the final model on MPA concentrations alone. This factor significantly influenced MPA clearance. A final model with better performance with regard to the precision of estimates and graphical analysis was obtained with the combined MPA and MPAG data. In table V, the final model parameters including co-variates are summarized. The introduction of an enterohepatic cycle (a non-null value for k_{41}) significantly improved the model ($\Delta OFV = 58$), but in the final combined model this was retained only in the case of sirolimus co-medication: the value of k_{41} was close to 0 (0.0004) in the case of ciclosporin co-medication (see equation 13). The AST/ALT ratio significantly influenced the MPA phenol-glucuronidation (CL) when introduced in the model as a categorical parameter as shown in equation 14, whereas the GFR estimated by both the Cockroft-Gault and Nankivell formulas linearly (see equation 11) significantly influenced the MPAG elimination rate constant, k_{40}. Only the Nankivell-calculated GFR was retained in the final model. A good estimation of all model parameters was obtained (standard error of estimates ≤41% of estimates) in the final model. Figures 3–5 show diagnostic plots of the final model's performance. The retained final model validation by bootstrapping and case deletion diagnostics followed by cross-validation gave satisfactory results. From the cross-validation, the RMSE and MRPE computed on OFVs were 24% and 18%, respectively. In addition, all parameters were included in the 15–60% confidence interval computed with the parameter values obtained from the 200 bootstraps. Finally, the predictive performance of the model was evaluated using a VPC. The population pharmacokinetic model was used to simulate 1000 hypothetical patients. The results are shown in figure 6. The overlap of the simulated and original distributions indicates the accuracy of the identified model.

Bayesian estimation of pharmacokinetic parameters from samples drawn at 0 hours (pre-dose), 1.25 hours and 2 hours after drug intake enabled the best prediction of the individual AUC_{12} with satisfactory accuracy and precision as compared either with the reference value obtained by using the trapezoidal method ($r^2 = 0.93$, MRPE = –0.4%, rRMSE = 12.4%) or with the Bayesian estimator computed from all samples ($r^2 = 0.96$, MRPE = 0.52%,

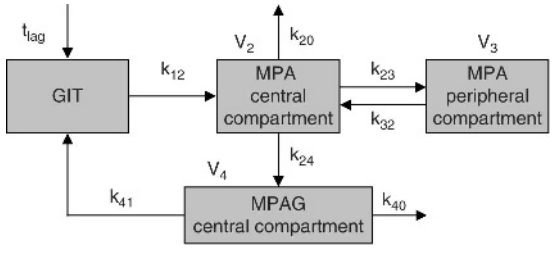

Fig. 3. Schematic diagram of the two-compartment model plus a metabolite compartment used to describe mycophenolic acid (MPA) and mycophenolic acid glucuronide (MPAG) pharmacokinetics. **GIT** = gastrointestinal tract; **k_{xy}** = intercompartmental transfer rate constant; **t_{lag}** = lag time; **V_2** = volume of distribution of the central compartment of MPA; **V_3** = volume of distribution of the peripheral compartment of MPA; **V_4** = volume of distribution of the central compartment of MPAG.

Table IV. Population pharmacokinetic analysis for the combined mycophenolic acid (MPA) and mycophenolic acid glucuronide (MPAG) plasma concentrations: structural model characteristics (n = 27 + 27 + 24)[a]

Parameter	Estimate [CV%][b]	IIV (estimate [CV%][b])	IOV (estimate [CV%][b])
$\theta_{t_{lag}}$ (h)	0.3 [8.9]		
$\theta_{k_{12}}$ (h^{-1})	1.9 [26]	17 [48]	58 [35]
$\theta_{V_2/F}$ (L)	14.0 [13]	18 [44]	21 [40]
$\theta_{V_3/F}$ (L)	248 [48]		
θ_{V_4/f_m} (L)	5 [23]	15 [39]	
$\theta_{CL/F}$ (L/h)	7 [7.2]	5.6 [29]	67 [26]
$\theta_{Q/F}$ (L/h)	24.3 [12]	27 [41]	
$\theta_{k_{20}}$ (h^{-1})	0.41 [27]		
$\theta_{k_{40}}$ (h^{-1})	0.21 [46]	43 [32]	9 [51]
$\theta_{k_{41}}$ (h^{-1})	0.06 [49]		62 [52]
$\varepsilon_{prop\ MPA}$ (%)	0.4 [14]		
$\varepsilon_{prop\ MPAG}$ (%)	0.2 [57]		
$\varepsilon_{add\ MPA}$ (μg/mL) [SD]	0.1 [102]		

a n = 27 in period I and II, and n = 24 in period III (see Patient Characteristics and Study Design section).

b CV% represents the precision of the estimate.

ε_{add} = additive error; ε_{prop} = proportional error; θ = population parameter; **CL**/**F** = apparent oral clearance; **CV** = coefficient of variation; f_m = fraction of the MPA dose converted to MPAG; **IIV** = interindividual variability; **IOV** = interoccasion (intra-individual) variability; k_{xy} = intercompartmental transfer rate constant; **Q**/**F** = apparent intercompartmental clearance after oral administration; t_{lag} = lag time; V_2/F = apparent volume of distribution of the central compartment of MPA after oral administration; V_3/F = apparent volume of distribution of the peripheral compartment of MPA after oral administration; V_4/F = apparent volume of distribution of the central compartment of MPAG after oral administration.

rRMSE = 19%). With this model, none of the patients had an AUC_{12} lower than −20% or higher than +20% of the reference value. Our algorithm applied to the validation group, i.e. n = 13 + 12 + 12, performed much better than two previously proposed algorithms applied to our validation dataset (table VI). Figure 7 shows the linear regression between the observed (trapezoidal method) and predicted (Bayesian estimation and MLR) AUC_{12} in the validation group.

Discussion

The pharmacokinetics of MPA and MPAG were determined in kidney transplant recipients receiving mycophenolate mofetil 0.75 g twice daily. Plasma concentrations of MPA and MPAG were measured during one dosing interval on three different occasions: during the initial post-transplantation period when patients received ciclosporin as co-medication, and 60 days and 270 days after switching the co-medication from ciclosporin to sirolimus.

MLR models were developed to predict individual AUC_{12} values by using two or three sampling times within 2 hours following mycophenolate mofetil administration. As expected, model equations based on three sampling times in general performed better than those based on two sampling times.

Table V. Population pharmacokinetic analysis for the combined mycophenolic acid (MPA) and mycophenolic acid glucuronide (MPAG) plasma concentrations: final model characteristics (n = 27 + 27 + 24)[a]

Parameter	Estimate [CV%][b]	IIV (estimate [CV%][b])	IOV (estimate [CV%][b])	95% CI of parameter estimates from 200 bootstraps
$\theta_{t_{lag}}$ (h)	0.26 [7.4]			0.1, 0.3
$\theta_{k_{12}}$ (h^{-1})	1.83 [32]		62 [18]	1, 4
$\theta_{V_2/F}$ (L)	14.7 [22]	3.2 [12]	21 [31]	11, 16
$\theta_{V_3/F}$ (L)	250 [32]			122, 476
θ_{V_4/f_m} (L)	6.31 [17]			2, 15
$\theta_{CL/F}$ (L/h)	14.7 [11]		13 [26]	8, 28
$\theta_{Sir\ on\ k_{41}}$ (h^{-1})	0.10 [14]			0.03, 0.5
$\theta_{k_{20}}$ (h^{-1})	0.36 [23]			0.08, 1.7
$\theta_{Q/F}$ (L/h)	21.1 [0.8]	17 [8]		12, 29
$\theta_{k_{20}}$ (h^{-1})	0.36 [23]			0.03, 1.6
$\theta_{k_{40}}$ (h^{-1})	0.008 [41]	2 [16]	5 [39]	0.001, 0.1
$\theta_{AST/ALT\ on\ CL/F}$	3.1 [33]			2.4, 6.2
$\theta_{GFR\ on\ k_{40}}$	0.12 [10.7]			0.07, 0.15
$\varepsilon_{prop\ MPA}$ (%)	0.41 [59]			0.25, 0.5
$\varepsilon_{prop\ MPAG}$ (%)	0.18 [0.4]			0.14, 0.4
$\varepsilon_{add\ MPA}$ (μg/mL) [SD]	0.19 [63]			0.16, 0.22

a n = 27 in period I and II, and n = 24 in period III (see Patient Characteristics and Study Design section).

b CV% represents the precision of the estimate.

ε_{add} = additive error; ε_{prop} = proportional error; θ = population parameter; **CL**/**F** = apparent oral clearance; **CV** = coefficient of variation; f_m = fraction of the MPA dose converted to MPAG; **GFR** = glomerular filtration rate calculated by the Nankivell formula; **IIV** = interindividual variability; **IOV** = interoccasion (intra-individual) variability; k_{xy} = intercompartmental transfer rate constant; **Q**/**F** = apparent intercompartmental clearance after oral administration; **Sir** = sirolimus; t_{lag} = lag-time; V_2/F = apparent volume of distribution of the central compartment of MPA after oral administration; V_3/F = apparent volume of distribution of the peripheral compartment of MPA after oral administration; V_4/F = apparent volume of distribution of the central compartment of MPAG after oral administration.

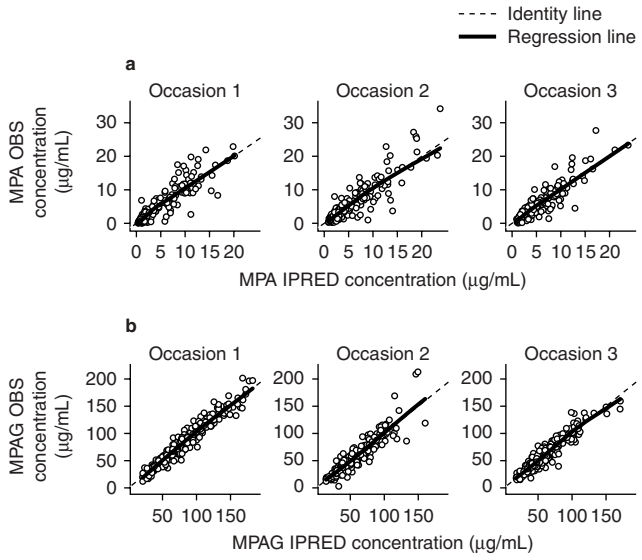

Fig. 4. Individual predicted (IPRED) vs observed (OBS) concentrations of (**a**) mycophenolic acid (MPA) and (**b**) mycophenolic acid glucuronide (MPAG) in the final population pharmacokinetic model. Occasions 1, 2 and 3 correspond to periods I, II and III (±7, ±9 and ±15 months after transplantation), respectively.

The MLR equation based on plasma concentrations of MPA obtained at 0 hours (pre-dose), 0.66 and 2 hours post-dose showed the best predictive performance, which was not very different from the predictive performance obtained with the Bayesian estimator. However, unlike MAP Bayesian estimation of the AUC_{12}, LSSs based on MLR require strict adherence to sampling times. Nevertheless, the MLR approach is interesting because it is easier to implement in routine practice than the Bayesian estimation method. Several LSSs based on MLR in stable renal transplant patients co-medicated with tacrolimus or ciclosporin have already been published.[25-28] First of all, the various model equations were based on sampling times ranging from 0 hours (pre-dose) to 12 hours post-dose, making some of them less practical as they would require a long hospital visit for the patient. Moreover, some of these model equations were neither internally nor externally validated. Recently, Figurski et al.[29] developed an MLR predictive LSS to estimate the AUC_{12} of MPA in stable renal transplant patients co-medicated with sirolimus or ciclosporin. The model equations were well validated and the sampling times were restricted, as in our case, to the 2-hour time period following mycophenolate mofetil administration. Their best model equation was based on sampling times at 0, 0.66 and 2 hours, exactly the same times selected for our MLR estimation of the MPA AUC_{12}.

This is the first report on MPA population pharmacokinetics in renal transplant patients on sirolimus co-medication and covering a post-transplantation period of up to 15 months. An enterohepatic elimination model with an absorption t_{lag} and first-order absorption for MPA, central and peripheral compartments for MPA, a central compartment for MPAG and first-order elimination of MPAG into the urine and into the GIT followed, in the case of sirolimus co-medication, by hydrolysis and reabsorption of MPA, best described the data. A population pharmacokinetic model was developed by using nonlinear mixed-effects modelling and validated by goodness-of-fit plots, precision of estimates, bootstrapping and simulation-based diagnostics.

Several population pharmacokinetic models have been described for MPA or MPA/MPAG in stable renal transplant patients.[21,22,30-35] Although the absorption following oral administration of mycophenolate mofetil is quite complex, in most cases a biexponential elimination model with first-order absorption and an absorption t_{lag} was selected to describe the data. Multiple peaks are often observed in the MPA plasma concentration-time profiles due to enterohepatic cycling.[30-32] MPAG excreted into the bile may be deconjugated back to MPA, which is subsequently reabsorbed. Biliary excretion of MPAG and subsequent distal reabsorption of MPA are likely to require several transport mechanisms, including organic anion transporters and multidrug resistance-associated protein 2 (MRP-2).[36] Ciclosporin has been shown to interrupt the enterohepatic cycling of MPA/MPAG by inhibiting MRP-2.[37,38] This could therefore be the reason why the

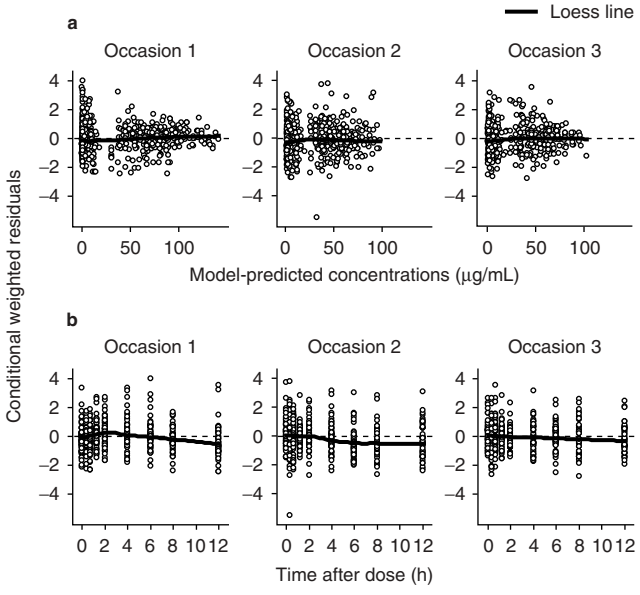

Fig. 5. Combined mycophenolic acid and mycophenolic acid glucuronide conditional weighted residuals (**a**) vs predicted concentrations and (**b**) vs time in the final population pharmacokinetic model. Occasions 1, 2 and 3 correspond to periods I, II and III (±7, ±9 and ±15 months after transplantation), respectively.

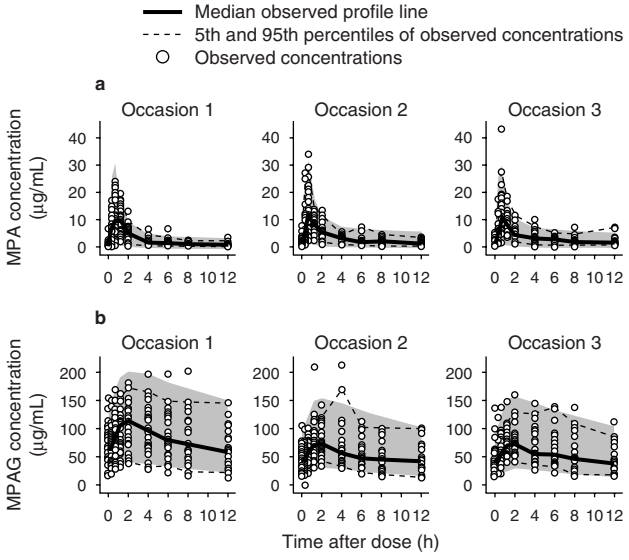

Fig. 6. Visual predictive check results of (**a**) mycophenolic acid (MPA) and (**b**) mycophenolic acid glucuronide (MPAG) concentrations. The shaded area shows the limits of the 5th and 95th percentiles of the simulated concentrations. Occasions 1, 2 and 3 correspond to periods I, II and III (±7, ±9 and ±15 months after transplantation), respectively.

structural pharmacokinetic model, which included a central compartment for MPAG and a first-order rate constant describing the excretion of MPAG into the GIT followed by hydrolysis and reabsorption of MPA, best fitted our data on both MPA and MPAG plasma concentrations (figure 2).

Inclusion of the following three co-variates in the population pharmacokinetic model significantly reduced the inter-individual, intra-individual or residual variability of certain pharmacokinetic parameters: AST/ALT on CL (i.e. MPA glucuronidation), GFR on k_{40} (MPAG renal excretion rate) and sirolimus co-medication on k_{41} (biliary excretion/hydrolysis of MPAG). Both the Cockroft-Gault and Nankivell estimations of the GFR showed a significant influence on MPAG renal excretion (i.e. k_{40}) during the co-variate inclusion process. However, only the GFR estimated by the Nankivell method was retained in the final model after the backward exclusion process, meaning that it affected the OFV more than the GFR estimated by the Cockroft-Gault method. In previous population pharmacokinetic studies of MPA or MPA/MPAG co-variates depending on liver function such as bilirubin, serum AST and ALT levels were not identified as significant co-variates. The influence of GFR on MPAG renal excretion is logical but has not been shown before in a population pharmacokinetic study, probably because MPAG measurements are usually not included in these models. Switching from ciclosporin to sirolimus as co-medication necessitates the inclusion of enterohepatic cycling in the model because ciclosporin effectively interrupts enterohepatic cycling of MPA/MPAG. It has previously been suggested that enterohepatic cycling may be more pronounced in the period immediately following transplantation as a possible explanation for the higher interindividual variability in MPA pharmacokinetics generally observed in the early post-transplantation period.[21] Our data show that enterohepatic cycling of MPA/MPAG is not significantly different at 9 months versus 15 months post-transplantation in patients receiving sirolimus as co-medication. Enterohepatic cycling in these patients, calculated as the ratio of k_{41} to $k_{41} + k_{40}$, and therefore representing the fraction of MPAG recycled in the body, was estimated to be approximately 46%.

Based on the final population pharmacokinetic model, MAP Bayesian estimation of individual AUC_{12} values was performed by using various combinations of three blood sampling times within 2 hours following mycophenolate mofetil administration. Plasma concentrations of MPA/MPAG obtained at 0, 1.25 and 2 hours resulted in the best predictive performance. The MRPE for this model was very small (–0.4%). The predictive performance of two previously published algorithms,[21,22] using Bayesian forecasting and based on three sampling times (0, 0.25 and 3 hours) to estimate individual AUC_{12} values in our studied patient population, was not as good as ours. There are two possible explanations why our algorithm performed better than the previously published Bayesian estimators. First, MPA and MPAG plasma concentrations were used to build our

Table VI. Comparison of the performance of two previously published Bayesian estimators with the Bayesian estimator based on the model developed in the present study

Study	Sampling times (h)	Estimation method	r^2	rRMSE (%)	MRPE (%) [95% CI]
Present	0, 1.25, 2	MEM/FOCEI	0.93	12.4	–0.4 [–0.9, 2.1]
Prémaud et al.[21]	0.33, 1, 3	ITS	0.42	74	27 [5, 30]
Le Guellec et al.[22]	0.33 ,1, 3	MEM/FO	0.54	48	33 [6, 47]

FO = first-order estimation method; **FOCEI** = first-order conditional estimation approach with interaction between parameters; **ITS** = iterative two-stage method; **MEM** = mixed-effects modelling; **MRPE** = mean relative prediction error; **rRMSE** = relative root mean square error.

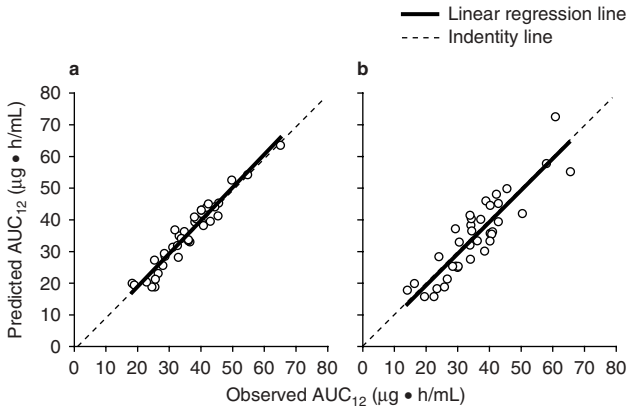

Fig. 7. Bayesian (**a**) and multiple linear regression (**b**) predicted area under the plasma concentration-time curve during one 12-hour dosing interval (AUC_{12}) vs the observed AUC_{12} computed by the trapezoidal rule.

model, whereas the previously published estimators were based on MPA plasma concentrations only. In addition, the validation group patients used to evaluate our Bayesian estimator had many common characteristics with the test group patients: same hospital, same clinical practice, same time post-transplant period, etc., whereas the previously published algorithms were evaluated on a really independent sample set.

While individualization of mycophenolate mofetil treatment in renal transplant patients based on target concentration intervention, particularly during the first 2 months post-transplantation, has been recommended by scientific societies and during consensus conferences, the use of a fixed-dose mycophenolate mofetil regimen in combination with calcineurin inhibitors and corticosteroids is still standard practice for the prevention of acute rejection. Le Meur et al.[39] showed that therapeutic drug monitoring using a Bayesian estimator of the MPA AUC_{12} based on three-point sampling reduced the risk of treatment failure and acute rejection in renal allograft recipients 12 months post-transplantation with no increase in adverse events. The results of the present study in renal transplant patients show that following administration of a mycophenolate mofetil 1.5 g/day fixed-dosage regimen, the AUC_{12} is located outside the therapeutic range in approximately 50% of the patients during the three study periods, suggesting the need for an individual dose adjustment.

Conclusion

Based on the results of the present study, the Bayesian estimator using MPA and MPAG plasma concentrations at 0, 1.25 and 2 hours would be an efficient tool to individualize mycophenolate mofetil dosage in renal transplant patients. Nevertheless, this approach should be validated in a randomized, prospective clinical trial.

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