Population pharmacokinetics of mycophenolic acid and dose optimization with limited sampling strategy in liver transplant children

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Population pharmacokinetic data for mycophenolic acid (MPA) have been reported in adult and paediatric kidney transplant recipients but not in paediatric liver transplant recipients.
- MPA AUC during a dosage interval (AUC(0,12 h) is routinely used to optimize mycophenolate mofetil (MMF) dosages in solid organ transplant recipients.

WHAT THIS STUDY ADDS

- This study describes MPA population pharmacokinetics in paediatric liver transplant recipients for the first time.
- The age of the children and the time elapsed since the transplantation were found to affect MPA pharmacokinetics significantly, mainly the apparent volume of distribution.
- The limited sampling strategy chosen to estimate individual MPA AUC(0,12 h) with concentrations measured 0, 1 and 4 h after MMF administration may be useful to optimize child care during the post transplantation period.

AIMS

The aims were to estimate the mycophenolic acid (MPA) population pharmacokinetic parameters in paediatric liver transplant recipients, to identify the factors affecting MPA pharmacokinetics and to develop a limited sampling strategy to estimate individual MPA AUC(0,12 h).

METHODS

Twenty-eight children, 1.1 to 18.0 years old, received oral mycophenolate mofetil (MMF) therapy combined with either tacrolimus ($n = 23$) or ciclosporin ($n = 5$). The population parameters were estimated from a model-building set of 16 intensive pharmacokinetic datasets obtained from 16 children. The data were analyzed by nonlinear mixed effect modelling, using a one compartment model with first order absorption and first order elimination and random effects on the absorption rate (k_a), the apparent volume of distribution (V/F) and apparent clearance (CL/F).

RESULTS

Two covariates, time since transplantation ($\leq$ and >6 months) and age affected MPA pharmacokinetics. k_a , estimated at 1.7 h^{-1} at age 8.7 years, exhibited large interindividual variability (308%). V/F , estimated at 64.7 l , increased about 2.3 times in children during the immediate post transplantation period. This increase was due to the increase in the unbound MPA fraction caused by the low albumin concentration. CL/F was estimated at 12.7 l h^{-1} . To estimate individual AUC(0,12 h), the pharmacokinetic parameters obtained with the final model, including covariates, were coded in Adapt II® software, using the Bayesian approach. The AUC(0,12 h) estimated from concentrations measured 0, 1 and 4 h after administration of MMF did not differ from reference values.

CONCLUSIONS

This study allowed the estimation of the population pharmacokinetic MPA parameters. A simple sampling procedure is suggested to help to optimize pediatric patient care.

Introduction

Mycophenolate mofetil (MMF) is the 2-morpholino-ethyl ester prodrug of mycophenolic acid (MPA). MMF has been approved for immunosuppressive therapy following a liver transplantation in adults but not in children. MMF is, however, being increasingly used for paediatric liver transplant recipients [1, 2]. MPA acts as a potent reversible inhibitor of inosine monophosphate dehydrogenase, the key enzyme in the *de novo* purine biosynthesis of proliferating T and B lymphocytes [3]. Its main advantage is its non nephrotoxic profile. After oral administration, MMF is rapidly and completely converted into MPA, its active metabolite. Ninety-seven per cent of MPA binds to plasma proteins (albumin) and this binding is affected by multiple factors, including hypoalbuminaemia, renal function and uraemia [4, 5]. MPA is mainly metabolized to the pharmacologically inactive MPA glucuronide (MPAG) by hepatic and renal glucuronosyl transferases (UGT) [4]. A rebound of the plasma MPA concentration occurs with many patients 6 to 12 h after oral administration of MMF, suggesting enterohepatic recycling of the drug [4]. Large inter- and intra-individual variations in the pharmacokinetic parameters of MPA have been described [6]. Therapeutic drug monitoring may help to reduce the risk of acute rejection, especially in the immediate post transplantation period. The risk of rejection has been reported to increase in adult and paediatric renal transplantation when the total MPA AUC(0,12 h) is less than 30 mg l⁻¹ h [7, 8]. Although the pharmacokinetic parameters of MPA are well established for adult renal transplant recipients [9, 10], paediatric renal transplant recipients [6] and adult liver transplant recipients [11–13], the data for paediatric liver transplant recipients remain limited. Full AUC(0,12 h) monitoring, however, is laborious, especially in children, it requires frequent blood sampling and is costly. Hence, some authors have suggested a limited sampling strategy (LSS) to calculate MPA AUC(0,12 h) in adult and paediatric renal transplant recipients [14–17] and in adult liver transplant recipients [18]. However, no such strategy has been suggested for the paediatric liver transplant population, as reviewed recently by Staatz & Tett [19].

The aims of this study were to investigate the population pharmacokinetics of MPA in children after liver transplantation, including inter- and intra-individual variability, and to suggest a limited sampling strategy for MPA AUC(0,12 h) estimation in such patients.

Methods

Patients and study design

Study population Between October 2006 and August 2010, 28 paediatric liver transplant recipients (14 boys and 14 girls) aged between 1.1 and 18.0 years (median 8.65 years) were treated with MMF in addition to standard

immunosuppressive therapy. The study protocol was approved by the 'Groupement Coopératif des Transplantations d'Ile de France' Review Board (GCIF) in June 2009. The indications for a liver transplantation were biliary atresia in 14 cases, fulminant hepatitis in eight, progressive familial intrahepatic cholestasis in three and Alagille syndrome, cystic fibrosis and Wilson disease in one case respectively. Median time since transplantation was 17.2 months (0.2–188.5 months). Immunosuppression to prevent rejection consisted in ciclosporin (median dose 3.1 mg kg⁻¹ day⁻¹, range 2.0–6.5 mg kg⁻¹ day⁻¹) for five children, tacrolimus (median dose 0.09 mg kg⁻¹ day⁻¹, range 0.01–0.21 mg kg⁻¹ day⁻¹) for 23 and steroids (median dose 0.50 mg kg⁻¹ day⁻¹, range 0.08–1.20 mg kg⁻¹ day⁻¹) for 14. Targeted ciclosporin and tacrolimus whole blood trough concentrations were 150–250 ng ml⁻¹ and 10–15 ng ml⁻¹, respectively during the month after the transplantation. MMF was introduced for the following indications: acute rejection, chronic rejection, enhancing the immunosuppression of tacrolimus or ciclosporin and reducing the doses of either ciclosporin or tacrolimus for patients threatened with impairment of renal function. The median starting dose of MMF was 380 mg m⁻² twice daily (equal to 13.0 mg kg⁻¹ twice daily) and ranged from 186 to 594 mg m⁻² twice daily (6.1 to 22.2 mg kg⁻¹ twice daily). The MPA dose adjustment was based on the AUC(0,12 h) estimated by non-compartmental analysis (WinNonLin Professional® version 5.1, Pharsight, Mountain View, California, USA) and the MMF dose was adjusted to target an MPA AUC(0,12 h) between 30 and 60 mg l⁻¹ h [20]. These adjustments were included in the follow-up of all children as routine clinical care. The treatment of acute rejection consisted of the administration of steroid boluses. The dose was 5 mg kg⁻¹ for 3 days and was tapered to 1.25 mg kg⁻¹ over the following 4 days. In addition, the tacrolimus or ciclosporin dose was increased in order to raise the trough concentration and MMF was introduced into the immunosuppressive regimen.

Blood samples were collected in vials containing ethylene diamine tetra-acetate (EDTA) for total and unbound MPA and MPAG assays in plasma at the following times during a 12 h dosing interval: before the morning dose (C₀) and 0.5, 1, 2, 4, 6 and 8 h after, in order to estimate the pharmacokinetic parameters by non compartmental analysis (see below) and to adjust the dose regimen of MMF [21]. For these assays, samples were rapidly centrifuged, and the plasma was kept frozen at –20°C until analysis. Standard laboratory tests to assess liver and kidney function were measured routinely. The body surface area was calculated in children using the following formula: Body surface area (m²) = (4 × weight (kg) + 7)/(weight (kg) + 90) [22] and creatinine clearance was estimated using the Schwartz formula [23]. The concentration vs. time data initially available from 16 patients were used as a model-building dataset (16 patients undergoing intensive pharmacokinetic sampling) and the data from the 26 patients

who were enrolled later (26 intensive pharmacokinetic data sets) were used as a model-validation dataset.

Drug assays

Ciclosporin and tacrolimus were respectively assayed in whole blood by antibody-conjugated magnetic immunoassay (ACMIA, Xpand Dimension, Siemens, Paris-La Défense, Paris, France) and enzyme-multiplied immuno-technique method (EMIT, Cobas Mira Plus, Roche, Meylan, France). The total and unbound concentrations of MPA and total MPAG in plasma were measured according to validated reverse-phase high-performance liquid chromatography (HPLC) methods, using Lichrosphere 100 RP 18 columns. The mobile phase consisted of 20:80 (v/v) acetonitrile–orthophosphoric acid buffer, 45:55 (v/v) acetonitrile–orthophosphoric buffer and 45:55 (v/v) acetonitrile–phosphate buffer for MPAG, total and unbound MPA, respectively. We assayed the MPAG after protein precipitation, total MPA by solid-liquid extraction on Bond-Elut® columns and unbound MPA in ultrafiltrate after centrifugation of plasma in Centrifree® devices. Ultra-violet detection was set at 216 nm. Within-day and between-day coefficients of variation were below 15%. The limits of quantification were 0.5, 0.0025 and 10 mg l⁻¹ for total MPA, unbound MPA and MPAG, respectively. Our laboratory participates in a programme of external quality controls for MPA assays (DW Holt, Cardiff Bioanalytical Services Ltd, UK).

Population pharmacokinetic modelling of total MPA

The data from 16 patients were used to estimate the population parameters of total MPA. Population pharmacokinetic modelling was performed using WinNonMix®, version 2.0.1 (Pharsight Corporation, Mountain View, CA, USA). A one compartment model at steady-state was used to describe the total MPA concentrations and was parameterized with the apparent volume of distribution (V/F), the first order absorption rate constant (k_a) and the apparent clearance (CL/F). Residual error was modelled on a proportional error model. An exponential random effect model was chosen to describe interindividual variability: $\theta_i = \theta \exp(\eta_i)$ where θ is the population mean vector of the pharmacokinetic parameters and η_i , the random effect vector. Random effects were assumed to follow a normal distribution with a zero mean and variance matrix Ω . In this study, a diagonal variance matrix Ω was chosen. The goodness of fit plots were examined for each model (observed vs. predicted population and individual concentrations and weighted residuals vs. predicted concentrations and vs. time).

The covariates tested were demographic characteristics (age, weight, body surface area and gender), plasma or serum chemistry parameters (serum creatinine, urea nitrogen, creatinine clearance, albumin, liver enzymes (alanine amino transferase [ALT] and aspartate amino transferase

[AST]), bilirubin concentration, factor V, haemoglobin, blood cell counts, neutrophils, platelets and haematocrit) and parameters related to transplantation (tacrolimus, ciclosporin or prednisone co-administration, the donor's age, acute or chronic rejection, the time elapsed since the transplantation, the time since the initiation of MMF and its dosage). Continuous variables were centred on their median. The effects of covariates were tested for each individual parameter (correlation test for continuous variables and ANOVA for categorical variables) using Statgraphics version 5.1 (Manugistics, Inc. Rockville, Maryland, USA). The covariates found to have a significant effect ($P < 0.05$) were then evaluated in the population analysis. The effect of a covariate was assessed by the likelihood ratio test. In the forward inclusion process, a covariate was retained in the model if there was a decrease greater than 3.84 in the objective function ($P < 0.05$, 1 degree of freedom) and if there was a decrease in the interindividual variability of the associated pharmacokinetic parameter. From the best model including covariates, a backward elimination procedure was then used to establish whether all the covariates selected should remain in the final model. When the withdrawal of a covariate ($P < 0.05$) significantly increased the log-likelihood (>3.84), that covariate was retained in the model.

The predictive performance of the model was evaluated using a visual predictive check. The population pharmacokinetic model was used to simulate 1000 hypothetical patients. The distribution (median and 10th and 90th percentiles) of the simulated concentration vs. time curves was compared with the total MPA concentrations observed in the model-validation set.

Bayesian methodology and limited sampling strategy

As WinNonMix® was not designed for the Bayesian approach and could not be used to estimate the individual AUC(0,12 h) for the children in the model-validation set, we decided to rewrite the final model for use with Adapt® II software [24]. Population pharmacokinetic parameters, including the time elapsed since transplantation and the age of the children, were coded in Adapt® II. The volume of the central compartment and the absorption rate were modelled as functions of the covariates. The individual AUC(0,12 h) of the patients in the model validation set (26 intensive pharmacokinetic data sets) were estimated using the final model and were compared, by the Bland–Altman test, with the corresponding values obtained by non-compartmental analysis (reference AUCs).

The reason for the sampling strategy used was to select the plasma concentrations that would be the most sensitive to differences in the absorption rate, volume of distribution and elimination rate. The performances of the limited sampling strategy (LSS) candidates for AUC(0,12 h) estimation were assessed for the model-validation set by comparing, using the Bland–Altman test, the individual

AUC(0,12 h) estimated by the LSS candidate with those obtained by non-compartmental analysis of all the data (reference AUCs). Three or four samples were tested at the following times after drug intake: either 0–0.5–2, 0–1–2, 0–2–4, 1–2–4 and 0–1–4 h or 0–0.5–1–2, 0–1–2–4 and 0–0.5–2–4 h.

Non-compartmental pharmacokinetic parameters

Pharmacokinetic parameters for unbound MPA, MPAG and the corresponding total MPA AUC(0,12 h) were estimated by a non-compartmental method, using the WinNonLin Professional® version 5.1 (Pharsight, Mountain View, California, USA). The AUC(0,12 h) was calculated using the linear up/log down trapezoidal rule. The ratio of MPAG AUC(0,12 h) : total MPA AUC(0,12 h) was calculated and the percentage of unbound MPA (the unbound MPA fraction, f_u) was determined for each patient by dividing the unbound MPA AUC(0,12 h) by the total MPA AUC(0,12 h) ($\times 100$). These parameters were analyzed depending on the post transplantation time, with a cut-off point of 6 months [1]. Statistical analysis was performed using GraphPad InStat® 3.05 (version 2000, GraphPad Software, San Diego, California, USA). Group comparisons were performed with the Mann-Whitney U-test. $P < 0.05$ was considered significant.

Results

Study population

The main characteristics of the study population are listed in Table 1 for the 16 model building and the 12 model validation children. One child had significant renal dysfunction (serum creatinine $364 \mu\text{mol l}^{-1}$ and creatinine clearance 17 ml min^{-1}). Ten children presented signs of acute rejection and seven of chronic rejection. Unbound MPA AUC(0,12 h) corresponding to total MPA AUC(0,12 h) below $30 \text{ mg l}^{-1} \text{ h}$, between 30 and $60 \text{ mg l}^{-1} \text{ h}$ and above

$60 \text{ mg l}^{-1} \text{ h}$, ranged from 0.05 to $0.95 \text{ mg l}^{-1} \text{ h}$, 0.14 to $1.22 \text{ mg l}^{-1} \text{ h}$ and 0.95 to $1.16 \text{ mg l}^{-1} \text{ h}$ respectively.

Population pharmacokinetics of MPA

Basic model The MPA concentrations were adequately described by a one compartment model with first order absorption and first order elimination. The population parameter estimates, their relative standard error of estimation (RSE %) and their interindividual variability for this basic model are shown in Table 2.

Covariate model building In the final population model, the age of the children affected the k_a and the time elapsed since the transplantation affected the V/F (immediate post transplantation period, ≤ 6 months, stable post transplantation period, > 6 months). The equations were: $k_{ai} = (k_{aTV} - \beta_{ka}^{age} (\text{age}_i/8.65)) \times \exp(\eta_{kai})$, where 8.65 (years) is the median age, TV is a typical value and $V/F_i = (V/F$

Table 2

Population pharmacokinetic parameters of mycophenolic acid (MPA) (estimates and relative standard errors of estimation, RSE) for the basic model and for the final model

Parameters	Basic model Estimate	RSE (%)	Final model Estimate	RSE (%)
k_a (h^{-1})	1.91	15.0	3.9	3.8
β_{ka}^{age}	–	–	2.2	3.7
CL/F (l h^{-1})	14.3	12.6	12.7	10.8
V/F (l)	92.0	16.8	64.7	13.2
$\beta_{V/F}^{time_{V/F}}$	–	–	2.3	26.3
ω_{ka} (%)	347.9	57.9*	308.4	68.7*
$\omega_{CL/F}$ (%)	43.1	60.8*	28.4	96.8*
$\omega_{V/F}$ (%)	59.2	88.9*	41.8	81.1*
σ (%)	62.3	30.0*	59.6	36.9*

*RSE for ω_{ka} , $\omega_{CL/F}$, $\omega_{V/F}$ and σ^2 . $\dagger V/F$ was 2.3 times higher in the immediate post transplantation period (≤ 6 months, $\beta_{V/F}^{time_{V/F}}$ ^ time post transplantation = 2.3) than in the stable post transplantation period (> 6 months, $\beta_{V/F}^{time_{V/F}}$ ^ time post transplantation = 1).

Table 1

Characteristics of the 28 patients included in the population pharmacokinetic analysis (median and range)

	Model building patients Median (range)	Model validation patients Median (range)
Number of patients	16	12
Age (years)	8.7 (1.1–15.2)	11.1 (2.2–18.0)
Weight (kg)	23.8 (9.3–49.2)	36 (13.5–63.2)
Body surface area (m^2)	0.9 (0.44–1.46)	1.22 (0.59–1.69)
ASAT (IU l^{-1})	64 (28–310)	70 (15–708)
ALAT (IU l^{-1})	93 (24–495)	100 (4–578)
Total bilirubin ($\mu\text{mol l}^{-1}$)	20 (4–263)	11 (6–599)
Serum creatinine ($\mu\text{mol l}^{-1}$)	37 (18–364)	57 (30–89)
Creatinine clearance (ml min^{-1})	155 (17–296)	118 (46–175)
Serum albumin (g l^{-1})	31.7 (17.2–35.0)	34.3 (39.0–54.7)

Figure 1

Observed MPA concentrations (open circles in patients in the early post transplantation period (≤ 6 months) and solid circles in patients in the stable post transplantation period (> 6 months) and predicted population concentrations for median age (8.65 years) in patients in the early post transplantation period (≤ 6 months, dashed line) and in the stable post transplantation period (> 6 months, continuous line)

$F_{TV} \times \beta_{V/F}^{\text{time post transplantation}} \times \exp(\eta_{V/F})$, when the time post transplantation was 0 if the patient was in the stable post transplantation period and 1 if the patient was in the immediate post transplantation period. All the factors previously tested as covariates on MPA CL/F during the forward inclusion process were not found to improve the model significantly and were not retained after the backward elimination procedure. The population parameters of this final model and their relative standard errors of estimation are given in Table 2. The absorption rate constant was estimated at 1.7 h^{-1} when the age was 8.65 years, with large interindividual variability of 308%. The apparent volume of distribution was estimated at 64.7 l and increased about 2.3 times (148.8 l) in children during the immediate post transplantation period (≤ 6 months) compared with the stable post transplantation period (> 6 months). The interindividual variability of the apparent volume of distribution was 41.8%. Interestingly, the interindividual variability for apparent clearance was very low, at 28.4%. CL/F was estimated at 12.7 l h^{-1} and the residual variability at 59.6%. Figure 1 shows the model-predicted concentrations and the observed concentrations vs. time at age 8.65 years, in the immediate post transplantation period (≤ 6 months) and the stable post transplantation period (> 6 months).

Effect of time since transplantation on MPA exposure

To illustrate further the influence of the time elapsed since the transplantation on MPA exposure, we compared the values of the pharmacokinetic parameters and the standard laboratory tests of children in the immediate post transplantation period (≤ 6 months, $n = 7$) with the values

of children in the stable post transplantation period (> 6 months, $n = 9$). The median albumin concentration was lower in the immediate than in the stable post transplantation period (26.8 vs. 33.0 g l^{-1} , $P = 0.0361$), resulting in a significantly higher unbound : total MPA ratio (2.8 vs. 1.0%, $P = 0.0496$) and significantly higher CL/F (16.0 vs. 11.2 l h^{-1} , $P = 0.0289$, Table 3). V/F was approximately 2.3 times higher in the immediate than in the stable post transplantation period (148.8 vs. 61.6 l, $P = 0.0095$, Table 3). Figure 2B shows that V/F was positively correlated with the unbound MPA fraction in both the immediate and stable post transplantation periods ($P < 0.0001$). The albumin concentration also correlated negatively with the unbound MPA fraction (Figure 2A, $P = 0.0003$). No difference was seen between the MPAG AUC : total MPA AUC ratios for the two periods (15.7 vs. 14.2, $P = 0.2315$, Table 3).

Model validation and optimal sampling times for AUC(0,12 h) estimation

Twenty-six intensive sets of pharmacokinetic data were obtained from 12 children and included in the model validation set, as several MPA pharmacokinetic studies were performed in the children in order to optimize the MMF dose. The goodness of fit plots of observed vs. population or individually predicted concentrations of MPA of the model and weighted residuals vs. predicted concentrations and vs. time (Figure 3) were evaluated and showed no structural bias. The final model was evaluated by visual predictive check (Figure 4). Less than 10% of the observed data were found to be outside the prediction interval of 90% for each time point, showing that the model adequately described the MPA concentrations observed over time. The individual MPA AUC(0,12 h) estimated with WinNonMix® or Adapt® II software were similar and close to the non-compartmental AUCs.

The three plasma samples collected before MPA administration and 1 and 4 h afterwards gave the best performances, as demonstrated by their correlation with the non-compartmental AUCs (Figure 5A, Pearson $r^2 = 0.9041$, $P < 0.0001$) and Bland-Altman plot (Figure 5B). Figure 5 shows that the model underestimated the MPA AUC(0,12 h) at values of approximately 30 $\text{mg l}^{-1} \text{ h}$.

Discussion

This study describes the population pharmacokinetics of MPA in paediatric liver transplant recipients for the first time as far as we know. Inter and intra-individual variabilities were taken into account in the model, because the age of the children and the time elapsed since the transplantation were shown to affect MPA exposure. In addition, our results show that the schedule involving three sampling times, 0 (pre dose), 1 and 4 h post dose, accurately predicts the plasma AUC(0,12 h) of MPA and might be very useful to optimize the MPA regimen in children after liver transplantation.

Table 3

Pharmacokinetic and serum chemistry parameters depending on the time post transplantation (median and range). MPA unbound fraction and MPAG AUC : total MPA AUC ratio were calculated by non-compartmental analysis, V/F and CL/F by population analysis

	≤6 months (n = 7)	>6 months (n = 9)	P value
Age (years)	9.0 (1.6–15.1)	8.0 (1.1–15.2)	NS
Weight (kg)	29.4 (10.0–47.0)	21.7 (9.3–49.2)	NS
f_u (%) (unbound MPA AUC : total MPA AUC ratio)	2.8 (1.2–8.5)	1.0 (0.3–3.8)	0.0496
MPAG AUC : total MPA AUC ratio	15.7 (7.9–72.6)	14.2 (7.6–39.4)	NS
V/F MPA ($l\ h^{-1}$)	148.8 (71.5–164.4)	61.6 (58.5–164.4)	0.0095
V/F MPA ($l\ h^{-1}\ kg^{-1}$)	6.2 (4.7–11.2)	3.3 (1.3–5.5)	0.0007
CL/F MPA ($l\ h^{-1}$)	16.0 (10.0–70.7)	11.2 (8.0–16.7)	0.0289
CL/F MPA ($ml\ min^{-1}$)	267 (167–1178)	187 (133–278)	0.0289
Serum albumin ($g\ l^{-1}$)	26.8 (17.2–33.0)	33 (26.9–35.0)	0.0361
Creatinine clearance ($ml\ min^{-1}$)	162 (17–296)	149 (99–205)	NS

f_u , MPA unbound fraction; V/F , apparent volume of distribution; CL/F , apparent clearance; NS, non significant.

Figure 2

(A) Relationship between MPA unbound fraction (f_u) and albumin concentration. (B) Relationship between V/F and MPA unbound fraction (f_u) in both the immediate and the stable post transplantation period ($P < 0.0001$). The straight line indicates linear regression. ≤ 6 months (open circles); > 6 months (solid circles)

Unlike the previous studies with adult and paediatric kidney transplant recipients, MPA pharmacokinetics were found to be characterized better by a single rather than a two compartmental model [16, 19, 25] with first order absorption and first order elimination and with random effects on the absorption rate (k_a), the apparent volume of

distribution (V/F) and apparent clearance (CL/F). Estimates of k_a , V/F and CL/F were in the same range as those previously reported in adult and paediatric kidney transplant recipients [16, 26, 27]. As previously described [4], inter-individual variability in the MPA absorption rate constant k_a was high. The administration of proton pump inhibitors with MMF was reported to decrease MPA AUC(0,12 h) [28]. However, since the proton pump inhibitors were administered 2 h after MMF, no interaction between the inhibitors and MMF was expected. A decrease in the absorption rate of MPA with increasing age was clearly demonstrated and may be due to the immaturity of the P-glycoprotein [29] which is probably involved in the intestinal absorption of MPA [30].

Great variability was observed in the apparent volume of distribution. V/F depends on the unbound fraction in plasma (f_u) and in tissue (f_{uT}), the volume of tissue (V_T) and the volume of plasma (V_P) as follows: $V/F = f_u/f_{uT} \times V_T + V_P$. For all drugs with a V/F above 30 l, such as MPA, a lipophilic drug ($\log P = 2.8$), V_P has only a minor effect on V/F and changes in f_u therefore directly reflect changes in V/F [31]. Consequently, the decrease in V/F during the stable post transplantation period probably results from the decrease in the unbound MPA fraction due to the normalization of the albumin and bilirubin concentrations as a consequence of liver function improvement [4, 5, 27]. Impaired renal function has also been reported to increase the unbound MPA fraction, because the increased MPAG concentration competes with MPA for the albumin binding sites [32]. Creatinine clearance remained unchanged over time in our population. Like most acidic drugs with a low hepatic extraction ratio (0.2 for MPA [33]), MPA CL/F is mostly related to either the albumin concentration or intrinsic clearance. Since most of the children in our series had received grafts from adult donors with mature UGTs, it is reasonable to think that changes in intrinsic clearance are unlikely. The greater decrease in MPA CL/F observed in the stable than in the immediate post-transplantation

Figure 3

Goodness of fit plots of observed vs. population predicted concentrations (A) and vs. individually predicted concentrations (B) and weighted residuals vs. predicted concentrations and vs. time of MPA (C and D). (A) Predicted (I) (—○—); Unity (—); (B) Predicted (P) (—○—); Unity (—); (C) Weighted Residual (P) (—○—); (D) Weighted Residual (P) (—○—)

Figure 4

Visual predictive check results of mycophenolic acid (MPA) concentrations performed on 1000 simulations. Dashed line shows the median and solid lines the 90th percentile interval. Less than 10% of the observed data were found to be outside the prediction interval of 90% for each time point. Predicted median (.....); Prediction interval 90% (—); Observed data (○)

period probably results from the decrease observed in the unbound MPA fraction. The variability in CL/F in our population was close to that reported for adult renal transplant recipients (36%) [27], but much lower than that reported for paediatric renal transplant recipients (66.2%) [16]. Other factors previously identified among adult liver transplant recipients, such as impaired renal function or bilirubin concentrations, were not found to have any effect on pharmacokinetic variability. However, the number of children included in this study was limited; only one child had marked renal dysfunction and only two had severe cholestasis.

Our model displayed great residual variability, probably due to the enterohepatic recycling occurring in some children and to the irregular individual concentration vs. time profiles. De Winter *et al.* [33] described a population pharmacokinetic model including the protein binding of both MPA and MPAG and the enterohepatic recycling of MPA with very long run times (55 h) due to the complexity of the model used [33]. No secondary concentration peak was

Figure 5

Relationship (A) and Bland-Altman plot (B) between AUCs calculated by trapezoidal rule (reference AUC(0,12 h)) or Bayesian estimation AUC(0,12 h) based on times 0, 1 and 4 h

seen in most of our patients, probably because of the absence of the gallbladder and the continuous secretion of bile into the small intestine [34, 35]. The absence of enterohepatic recycling explains why no difference in MPA exposure was detected in our study, regardless of the comedication with tacrolimus and ciclosporin [36]. Consequently, the enterohepatic recycling of MPA was not taken into account in the present model.

This study shows that most of the interindividual variability is due to k_a and V/F , which argues in favour of therapeutic drug monitoring of MPA using AUC(0,12 h) rather than the pre dose concentration, in paediatric liver transplant recipients. In this connection, data obtained from adult and paediatric transplant recipients have shown that the AUC(0,12 h) for MPA is strongly related to the risk of acute rejection [6, 7] and is a better predictor of outcome than the pre dose concentration [37, 38]. In the study by Weber *et al.* [8], an MPA AUC(0,12 h) above 33.8 mg l⁻¹ h in the initial post transplantation phase was related to a reduced risk of acute rejection in paediatric renal transplant recipients. In addition, exposure to unbound MPA was shown to have greater predictive value than total MPA AUC(0,12 h) as a risk factor for leukopenia, with a cut-off value in the first 1–3 weeks of 0.4 mg h l⁻¹. However, this last approach, requiring ultrafiltration on Centrifree® devices, is time consuming, costly and not appropriate for

routine dose optimization. Some algorithms or Bayesian approaches have been validated for the estimation of a total MPA exposure in paediatric renal transplantation, with two or three sampling times during the first 2 or 4 h after MMF intake [16, 19, 39–42]. Our results show that the schedule involving three sampling times (0, 1 and 4 h) accurately predicts the AUC(0,12 h) of MPA in plasma, with an r^2 of 0.90 in paediatric liver transplant recipients. We noticed a slight underestimation of MPA AUC(0,12 h) in the 30 mg l⁻¹ h range, but it had no clinical consequences, because in our institution, the target AUC(0,12 h) is 45 mg l⁻¹ h in order to avoid graft rejection.

In conclusion, we believe this is the first description of the population pharmacokinetics of MPA in paediatric liver transplant recipients. The age of the children and the time elapsed since the transplantation were found to affect significantly MPA AUC(0,12 h). An excellent correlation was demonstrated between the reference AUC(0,12 h) and the AUC(0,12 h) calculated with the Bayesian approach. The suggested limited sampling strategy may be useful to estimate the individual MPA AUC(0,12 h) and to optimize the MMF dose required to achieve a target MPA AUC(0,12 h) between 30 and 60 mg l⁻¹ h, which is associated with a reduced risk of acute rejection in children.

Competing Interests

There are no competing interests to declare.

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