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The Pharmacokinetic-Pharmacodynamic Relationship for Total and Free Mycophenolic Acid in Pediatric Renal Transplant Recipients: A Report of the German Study Group on Mycophenolate Mofetil Therapy

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Abstract. It is currently being debated whether pharmacokinetic monitoring of mycophenolic acid (MPA), the active constituent of mycophenolate mofetil (MMF), can optimize MMF therapy after organ transplantation. This open-label longitudinal study in pediatric renal transplant recipients was designed to investigate the pharmacokinetic (PK)/pharmacodynamic relationship of total and free MPA and to establish PK values for the assessment of an individual's MPA PK parameters. Fifty-four children, aged 2.2 to 17.8 yr, on an immunosuppressive triple regimen consisting of cyclosporin A, prednisone, and MMF (600 mg/m^2 body surface area twice daily) were investigated 1 wk and 3 wk (initial phase) and 3 mo and 6 mo (stable phase) after renal transplantation. MPA was measured by reverse phase HPLC, free MPA by HPLC after separation by ultrafiltration. There was an association between the risk of acute rejection episodes and MPA-$AUC_{0\text{-}12}$ values or MPA predose levels; by receiver operating characteristic analysis, an $AUC_{0\text{-}12}$ of 33.8 mg $\times$ h/L in the initial phase posttransplant had a diagnostic sensitivity of 75% and a diagnostic specificity of 64% for discrimination of patients with acute rejections. The respective discrimination threshold for the MPA predose concentration was 1.2 mg/L with a sensitivity of 83% and a specificity of 64%. In contrast, high free, but not total, MPA-$AUC_{0\text{-}12}$ values were associated with an increased risk of the MMF-related side effects leukopenia and/or infections. These data indicate that therapeutic drug monitoring of MPA has the potential for optimization of MMF efficacy in this patient population by steering patients away from the low values of MPA PK variables that are associated with an increased rejection risk. For the assessment of the toxic risk of MMF regarding leukopenia and/or infections, measurement of free MPA appears to be more appropriate.

Three randomized, double-blinded, controlled trials in adult renal transplant recipients demonstrated that mycophenolate mofetil (MMF) is more effective than azathioprine or placebo in combination with cyclosporin A and corticosteroids for prevention of allograft rejection (1). MMF is now widely used for maintenance immunosuppressive therapy also in pediatric renal transplant recipients (2). However, data on the pharmacokinetics and pharmacodynamics of MMF in this patient population are still limited.

MMF is rapidly metabolized *in vivo* to its active constituent, mycophenolic acid (MPA), a reversible, uncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPDH). Inhibition of IMPDH-II in activated lymphocytes causes a reduction in intracellular guanine nucleotide pools and leads to an arrest of lymphocyte proliferation. MPA is extensively bound to albumin, with a range of protein binding of 97% to 99% in patients with normal renal and liver function (3–5). On the basis of *in vitro* investigations, free MPA concentrations are presumed to be responsible for its immunosuppressive action (4). The primary metabolite of MPA is the phenolic glucuronide 7–0–MPAG. Two further metabolites have been identified in humans, namely the acyl glucuronide (AcMPAG) and the phenolic glucoside of MPA (6). Of these three metabolites, only the acyl glucuronide is capable of inhibiting human IMPDH-II *in vitro* (7).

The pharmacokinetics of MPA shows large interindividual and intraindividual variability. Pharmacokinetic monitoring of MPA with the aim of optimizing the dosage of this drug to achieve adequate immunosuppression with minimized risk of graft rejection or toxicity has therefore been under investigation (8). In adult renal transplant recipients on cyclosporine,

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MMF, and steroids, pharmacokinetic/pharmacodynamic (PK/PD) relationships between MPA-AUC or predose MPA levels and the risk of acute rejection have been established on the basis of measurements by reverse-phase HPLC with ultraviolet detection (9,10).

The interpretation of the PK/PD relationship of MPA in different patient populations can be influenced by several factors, such as the nature of the organ transplanted, the age of the patient, use of concomitant immunosuppressive therapy, protein binding, the presence of active metabolites, and the assay used. The purposes of this investigation were therefore (*1*) to establish the PK/PD relationships for total and free MPA in pediatric renal transplant recipients on an immunosuppressive regimen with cyclosporin A, MMF, and corticosteroids and (2) to establish the therapeutic ranges for MPA-AUC_{0-12} values and predose MPA levels in this patient population.

Materials and Methods

Patients

This study was an open-label longitudinal evaluation of the pharmacokinetic-pharmacodynamic relationship for total and free MPA in pediatric renal transplant recipients. The inclusion and exclusion criteria have been described in previous reports (5,11). The analysis of the clinical results of this study is being published elsewhere (12). The present analysis of the PK/PD relationship involved 54 patients (33 boys) with a median age of 11.7 yr (range, 2.2 to 17.8 yr). All patients were white. Forty-four of 54 children had primary transplant function, ten patients had delayed graft function defined as the requirement for dialysis in the first 3 wk posttransplant. No graft loss occurred in the delayed graft function group. Immunosuppressive therapy consisted of methylprednisolone (300 mg/m^2 body surface area [BSA]) on the day of transplant surgery which was then tapered to 60 mg/m^2 for the first, 30 mg/m^2 for the second, 15 mg/m^2 for the third, 12 mg/m^2 for the fourth, 9 mg/m^2 for the fifth, and 6 mg/m^2 for the sixth week after transplantation and 4 mg/m^2 thereafter. Cyclosporin A (microemulsion formulation) was administered in a dose of 500 mg/m^2 per day given in two divided doses for 24 h, starting 6 h after surgery. Thereafter, doses (approximately 300 mg/m^2 BSA per d) were adjusted to achieve 12-h trough levels of 150 to 250 ng/ml, as measured by whole blood monoclonal fluorescence polarization immunoassay (mFPIA, Abbott, Chicago, IL), in the first 3 mo posttransplant; thereafter, 12-h trough levels of 100 to 200 ng/ml were targeted. The mean cyclosporine dose and the respective 12-h predose levels at the four pharmacokinetic sampling time points are listed in Table 1.

Dosage of MMF

MMF was administered orally in a dose of 600 mg/m^2 BSA twice a day up to a maximum of 2 g/d. This dose was based on a preliminary report of a dose-finding study in pediatric renal transplant recipients (13). If the dose could not be exactly administered by use of 250 mg capsules, MMF capsules were opened and the exact dose for each individual child was refilled into gelatin capsules comparable to those produced by the MMF manufacturer. BSA was calculated by the formula of Du Bois and Du Bois (14).

Pharmacokinetic Protocol

Patients were studied after informed (parental) consent was obtained. Blood samples for pharmacokinetic assessments were drawn on days 7 and 21 posttransplant (initial phase) and 3 and 6 mo posttransplant (stable phase). It was mandatory that all patients had at least 2 full days of the same MMF dose given twice a day before pharmacokinetic investigations. The study was performed under inpatient conditions, starting in the morning. Patients were required to fast from 10 p.m. the night before sampling until after the 75-min sample had been obtained on the following morning. Blood samples were collected at the following times: before dosing and 20 min, 40 min, 75 min, and 2, 4, 6, 8, and 12 h after dosing. The study protocol was approved by the local ethics committee of each contributing center. All blood samples were collected in tubes containing ethylenediaminetetraacetic acid as an anticoagulant. For determination of MPA, free MPA, and MPAG concentrations, plasma was separated and stored at −20°C until analysis.

Measurement of Total and Free MPA and MPAG

The procedure for the determination of total MPA and MPAG in plasma, as well as for the determination of the free concentration of MPA in plasma water has been described in detail elsewhere (5,15). The limit of quantification for free MPA (at 215 nm) was set at 10 μg/L because the imprecision at the detection limit of 5 μg/L was >20%. The within-day imprecision ranged from 6.5% (209.2 μg/L) to 11.6% (18.4 μg/L) and the between-days imprecision ranged from 7.2% (200.6 μg/L) to 14.6% (22.8 μg/L) for measurement of free MPA at 215 nm (19).

Pharmacokinetic Analyses

The following pharmacokinetic data for MPA, free MPA, and MPAG were determined: predose concentration (C_0), time to maximum concentration (t_{max} [h]), maximum concentration (C_{max} [mg/L]), area under the curve (AUC) from 0 to 12 h (mg × h/L) using the linear trapezoidal rule, and the evening predose (*i.e.*, the 12-h) concentration (C_{12} [mg/L]). An abbreviated three-point AUC based on the sampling times 0 min, 75 min, and 4 h (MPA-$AUC_{0,75\ min,4\ h}$) was calculated according to a previously published algorithm (20): estimated AUC = $11.8 + 3.71 \times C_0 + 1.33 \times C_{75\ min} + 3.9 \times C_{4\ h}$, where C_0 is the predose MPA concentration, and $C_{75\ min}$ and $C_{4\ h}$ are MPA concentrations 75 min and 4 h, respectively, after MMF dosing. In addition, a published algorithm based on an empiric equation using a limited sampling strategy up to 2 h postdose (MPA-AUC_{0-2}) was taken for the calculation of the full AUC (21). The pharmacokinetic analysis was performed using the computer program BiAS (Epsilon-Verlag Hochheim-Darmstadt, Germany).

Acute Rejection Episodes

The primary outcome criterion for the determination of the PK/PD relationship for MPA was the occurrence of acute rejection episodes over the 6-mo study period after transplantation. Fifteen of 54 patients experienced at least one acute rejection episode during the 6-mo study period; two of these patients had two rejection episodes, resulting in a total number of 17 acute rejection episodes. Twelve of 17 acute rejection episodes were biopsy-proven, and histologic examination and classification of a core biopsy was done according to the Banff criteria (16). If a biopsy was logistically impossible or clinically contraindicated, the diagnosis of "presumed rejection" was based on clinical judgment (supported by one or more of the following clinical findings: increased body temperature, graft swelling, graft tenderness, rise in serum creatinine level of more than 20% from the baseline level, or oliguria). The clinical diagnoses were made without any knowledge of the MPA concentration data. Acute rejection episodes were treated initially with high-dose intravenous corticosteroids (methylprednisolone, 400, 200, 200, and 100 mg/m^2 BSA on 4 con-

secutive days, respectively, or 300 mg/m^2 BSA for 3 to 5 d). If the rejection episode failed to respond to this therapy, treatment with OKT3 or antithymocyte globulin was started or patients were converted from cyclosporine to tacrolimus according to center practice. The acute rejection episodes occurred 27 d (range, 8 to 170 d) after renal transplantation; 14 of 17 rejections occurred within the first 70 d posttransplant.

Adverse Event Monitoring

Adverse events, defined as an abnormal change in physical signs, symptoms, or laboratory values whether or not deemed to be causally related with the study medication, were recorded throughout the study when reported by a patient or noted by an investigator. Thrombocytopenia was defined as a thrombocyte count $<150 \times 10^{12}$/L. Leukopenia was defined as a granulocyte count $<2000/\mu$l and graded according to its severity: mild (1600 to 1999 granulocytes); moderate (1000 to 1599 granulocytes) ($n = 5$); severe (500 to 999 granulocytes). Diarrhea was graded as follows: mild diarrhea ($n = 7$), transient diarrhea $\leq$2 d; moderate diarrhea ($n = 3$), tolerable diarrhea lasting $>$2 d; and severe diarrhea ($n = 1$), intolerable diarrhea requiring therapy. Infections were classified as moderate when requiring specific antibiotic or antiviral therapy and as severe when requiring hospitalization. The following infections were recorded: herpes labialis ($n = 5$), herpes zoster ($n = 1$), oral mucocutaneous candidiasis ($n = 1$), urinary tract infection ($n = 8$), cytomegalovirus (CMV)–pneumonia ($n = 1$), CMV-colitis ($n = 1$), bacterial septicemia ($n = 3$), pneumonia ($n = 1$), pharyngitis ($n = 1$), febrile viral infection ($n = 4$).

Statistical Analyses

The Shapiro-Wilks test was used to confirm normal distribution of data (17). Because some parameters were normally distributed and some were not, data in Table 1 and 2 are given as median (range). For comparison of more than two groups, one-way ANOVA on repeated measurements followed by all pairwise comparison (Student Newman-Keuls test) was used. Forward stepwise regression analysis was performed to assess which variables contribute independently to the prediction of MPA-AUC$_{0-12}$ values. Differences were considered to be statistically significant at a $P < 0.05$.

Receiver operating characteristic (ROC) plots of sensitivity *versus* 1-specificity were generated to determine whether a particular pharmacokinetic variable could discriminate patients with an acute rejection from those who experienced no rejection. Areas under the ROC curves and the 95% confidence interval (CI) limits were calculated using the method of Hanley and McNeil (18). The ROC curve analysis was carried out with Analyze-It version 1.44 (Analyze-It Software, Leeds, UK).

Results

Pharmacokinetic Results

In agreement with our previous finding, there was a large interindividual variation of PK data, despite the fact that all patients were receiving the same body surface–adjusted MMF dosage (Table 2). For example, MPA-AUC$_{0-12}$ values at 3 mo posttransplant ranged from 28.6 to 139 mg $\times$ h/L. The interindividual coefficient of variation was comparable in the initial (18%) and stable phase (22%) posttransplant. In an attempt to explain the variability in MPA-AUC$_{0-12}$ values, correlation analyses with clinical parameters were performed. At the PK sampling period 1 wk posttransplant, there were significant positive correlations between patient age, serum albumin, GFR, and the MPA-AUC$_{0-12}$ values (Table 3). However, none of these correlations remained significant at the three later PK sampling periods (Table 3). Because of the multiple interrelations between age, serum albumin, and GFR, forward stepwise regression analysis was performed to further assess which variables contribute independently to the prediction of MPA-AUC$_{0-12}$. MPA-AUC$_{0-12}$ at 1 wk posttransplant could be predicted from a linear combination of the independent variables age, GFR, and serum albumin ($r = 0.73$; $P < 0.0001$). Patients with MPA-AUC$_{0-12}$ levels at 1 wk posttransplant below the median of 36 mg $\times$ h/L tended to be younger (10.2 $\pm$ 0.8 *versus* 12.3 $\pm$ 0.6 yr; $P = 0.067$) and had significantly lower serum albumin levels (33.1 $\pm$ 1.1 *versus* 37.9 $\pm$ 1.5 g/L; $P < 0.03$) and significantly lower GFR (52.7 $\pm$ 6.7 *versus* 71.5 $\pm$ 6.4 ml/min per 1.73 m^2; $P < 0.05$) than those with MPA-AUC$_{0-12}$ above 36 mg $\times$ h/L. Hence, in the first week posttransplant, but not at later PK sampling periods, low MPA-AUC$_{0-12}$ values were associated with young age, low serum albumin levels, and decreased renal transplant function.

Whereas the PK parameters at 1 wk and 3 wk posttransplant were not significantly different, there was an increase of the PK parameters C_0 (132%), C_{12} (79%), and AUC$_{0-12}$ (89%) between the 3-wk and the 3-mo sampling period (Table 2). There was no further statistically significant increase of MPA PK parameters between the 3-mo and 6-mo sampling period, in agreement with our previous report in a smaller cohort of patients (11). In contrast to total MPA-AUC$_{0-12}$, the AUC$_{0-12}$ of free MPA did not change over time (Table 2), as reported previously (11).

Because a full MPA-AUC requires at least 8 blood samples during a 12-h dose interval and is therefore impractical in

Table 1. Cyclosporine dose and whole blood predose levels in 54 pediatric renal transplant recipients at the four pharmacokinetic sampling periods posttransplant[a]

Cyclosporine A	Pharmacokinetic Sampling Period			
	1 wk	3 wk	3 mo	6 mo
Dose (mg/kg per d)	7.85 (3.13 to 17.7)	6.98 (3.81 to 20.7)	5.90 (2.65 to 13.4)	5.70 (3.06 to 13.8)
Predose level (ng/ml)	185 (31 to 452)	193 (126 to 306)	172 (73 to 258)	144 (85 to 199)

[a] Data are median (range).

Table 2. Comparison of the pharmacokinetic (PK) parameters for MPA and free MPA at the four PK sampling periods posttransplant in 54 pediatric renal transplant recipients[a]

Parameter	1 wk		3 wk		3 mo		6 mo	
	MPA	Free MPA	MPA	Free MPA	MPA	Free MPA	MPA	Free MPA
C_0 (mg/L)	1.06[a] (0.11 to 4.13)	0.01[c] (<0.01 to 0.09)	0.97[a] (0.06 to 15.6)	0.01[c] (<0.01 to 0.15)	2.25[b] (0.35 to 11.2)	0.02[c] (<0.01 to 0.17)	2.92[b] (0.18 to 12.6)	0.02[c] (<0.01 to 0.11)
C_{12} (mg/L)	0.92[a] (0.11 to 6.96)	0.01[c] (<0.01 to 0.11)	0.92[a] (0.00 to 13.2)	0.01[c] (<0.01 to 0.10)	1.65[a] (0.28 to 6.89)	0.01[c] (<0.01 to 0.10)	2.31[b] (0.25 to 12.2)	0.02[c] (<0.01 to 0.22)
C_{max} (mg/L)	9.95[a] (1.50 to 42.3)	0.17[c] (0.03 to 0.61)	15.3[a] (3.53 to 45.7)	0.20[c] (0.03 to 0.65)	24.9[b] (7.20 to 53.5)	0.23[c] (0.08 to 0.61)	26.0[b] (9.40 to 52.5)	0.21[c] (0.07 to 0.57)
AUC_{0-12} (mg × h/L)	36.0[a] (3.12 to 95.0)	0.41[c] (0.07 to 1.58)	33.6[a] (12.8 to 89.1)	0.41[c] (0.13 to 1.19)	63.5[b] (28.6 to 139)	0.49[c] (0.17 to 1.40)	65.7[b] (21.3 to 117)	0.51[c] (0.18 to 1.34)

[a] Data are median (range). Values sharing common superscripts are not significantly different, whereas values without common superscripts are significantly different ($P < 0.05$).

Table 3. Patient age, serum albumin levels, and GFR at the four PK sampling periods posttransplant in 54 pediatric renal transplant recipients and the coefficients of correlation of these parameters with the respective MPA-AUC_{0-12} values[a]

Parameter	1 wk		3 wk		3 mo		6 mo	
	Mean ± SE (Range)	Correlation	Mean ± SE (Range)	Correlation	Mean ± SE (Range)	Correlation	Mean ± SE (Range)	Correlation
Age (yr)	11.1 ± 0.51 (3.17 to 16.0)	$r = 0.40$ $P < 0.005$	11.1 ± 0.51 (3.17 to 16.0)	$r = 0.11$ $P = 0.45$	11.3 ± 0.51 (3.42 to 16.3)	$r = 0.06$ $P = 0.73$	11.6 ± 0.51 (3.67 to 16.5)	$r = 0.03$ $P = 0.86$
Albumin (g/L)	35.4 ± 0.99 (24.0 to 51.0)	$r = 0.47$ $P < 0.005$	37.2 ± 0.84 (27.0 to 53.0)	$r = 0.09$ $P = 0.59$	41.8 ± 0.84 (23.0 to 47.0)	$r = 0.34$ $P = 0.052$	42.8 ± 0.62 (36.0 to 47.0)	$r = 0.31$ $P = 0.11$
GFR (mL/min per 1.73m^2)	62.6 ± 4.57 (9.0 to 133)	$r = 0.33$ $P < 0.04$	80.1 ± 4.34 (14.0 to 143)	$r = 0.12$ $P = 0.48$	84.8 ± 3.85 (39.0 to 139)	$r = 0.28$ $P = 0.09$	84.1 ± 4.23 (45.0 to 123)	$r = 0.30$ $P = 0.12$

[a] Data are mean ± SE (range).

clinical routine practice, we investigated whether a single time point MPA concentration or an abbreviated AUC derived from a limited number of samples correlate with the full MPA-AUC. Only a moderate correlation was observed between either the predose MPA trough concentration (C_0) or the 12-h evening trough concentration (C_{12}) and the full MPA-AUC_{0-12}. Although the C_0 concentration may have been taken after a more variable time interval subsequent to the previous dose than the C_{12} concentration that was sampled exactly 12 h after a supervised dose, the correlation of C_{12} with the full AUC was not superior to that of C_0 (Table 4). There was also only a moderate correlation between the MPA peak concentration (C_{max}) and the respective MPA-AUC_{0-12} (Table 4). Because MPA C_{max} increases during the first 3 mo posttransplant more markedly than the MPA predose concentrations (Table 2), the coefficients of correlation of MPA C_{max} with the respective full AUC in the stable phase posttransplant were higher and the coefficients of correlation of MPA predose levels were lower than in the initial phase posttransplant. The abbreviated MPA-$AUC_{0,75\ min,4\ h}$ gave a reasonable correlation with the full AUC that was somewhat superior to that seen between the abbreviated profile MPA-AUC_{0-2} and the full AUC (Table 4).

To provide the context for an assessment of an individual's PK parameter in relation to the pediatric population distribution, 5^{th} to 95^{th} percentiles for MPA-AUC_{0-12} and predose (C_{12}) levels were calculated (Figure 1).

PK/PD Analyses Regarding Acute Rejections

There were 17 acute rejection episodes in 54 patients during the 6-mo study period. Figure 1 shows the MPA-AUC_{0-12} and MPA-C_{12} values of the rejectors in relation to the respective percentiles of the whole study population; in the initial phase posttransplant, both the AUC_{0-12} and MPA-C_{12} values were distributed in the lower PK percentiles (Figure 1). Ten of fourteen patients with rejections in the initial phase posttransplant had MPA-AUC values below 33.8 mg × h/L, before they experienced an acute rejection episode. The comparison to those patients who neither suffered from an acute rejection nor an adverse event yielded a relative risk of acute rejection of 41% with total MPA-AUC_{0-12} values being below 33.8 mg × h/L compared with 14% with total MPA-AUC values being above 33.8 mg × h/L. The cyclosporine dose (rejectors, 7.5 [4.2 to 13] mg/kg per d; nonrejectors, 8.0 [3.1 to 14] mg/kg per d), and the 12-h predose whole blood levels (rejectors, 187 [76 to 330] mg/L; nonrejectors, 190 [88 to 331] mg/L) were not significantly different between the two groups (data are median [range] of the respective mean data calculated from the PK sampling time points at 1 wk and 3 wk posttransplant).

To establish which pharmacokinetic parameter is the best predictor for the risk of acute rejection, ROC curves were computed for each of the four PK parameters. For these calculations, the mean values from the 1-wk and 3-wk sampling time points were taken for each variable and patient. Diagnostic sensitivities (true positive) were calculated for each individual PK value as the fraction of those patients with an acute rejection who had a value below this discrimination threshold. The corresponding diagnostic specificities (false negatives) were calculated as the fraction of patients with no rejection episode who had a value below this decision value. The ROC plots of sensitivity *versus* 1-specificity for the PK variables AUC_{0-12} and C_{12} are shown in Figure 2, and the statistical comparison of the areas under the ROC curves is given in Table 5. C_{12}, AUC_{0-12}, and the two abbreviated AUC estimations were able to discriminate patients with acute rejections from patients with no rejection. An AUC_{0-12} of 33.8 mg × h/L had a diagnostic sensitivity of 75.0% and a diagnostic specificity of 64.3%. For the PK parameter C_{12}, a concentration of 1.2 mg/L gave a sensitivity of 83.3% and a specificity of 64.3%. In contrast, the PK variables derived from the measurements of free MPA were not effective for the discrimination of rejectors from nonrejectors (Table 5).

PK/PD Analyses Regarding Adverse Events

High free MPA-AUC_{0-12} values were associated with an increased risk of certain MMF-related side effects. Free MPA-AUC_{0-12} values of patients with infections and/or leukopenia

Table 4. Correlation of the MPA PK parameters, C_0, C_{12}, and C_{max}, and the abbreviated profiles, MPA-$AUC_{0,75min,4h}$ and MPA-AUC_{0-2}, with the full-time MPA-AUC_{0-12} at the four PK sampling periods posttransplant in 54 pediatric renal transplant recipients

PK Parameter	1 wk	3 wk	3 mo	6 mo	Entire Study Period
MPA C_0	$r = 0.65$ $P < 0.0001$	$r = 0.70$ $P < 0.0001$	$r = 0.59$ $P < 0.0001$	$r = 0.50$ $P < 0.01$	$r = 0.64$ $P < 0.0001$
MPA C_{12}	$r = 0.69$ $P < 0.0001$	$r = 0.56$ $P < 0.0001$	$r = 0.49$ $P < 0.01$	$r = 0.47$ $P < 0.01$	$r = 0.52$ $P < 0.0001$
MPA-C_{max}	$r = 0.53$ $P < 0.005$	$r = 0.52$ $P < 0.005$	$r = 0.68$ $P < 0.0001$	$r = 0.64$ $P < 0.0001$	$r = 0.71$ $P < 0.0001$
MPA-$AUC_{0,75min,4h}$	$r = 0.89$ $P < 0.0001$	$r = 0.86$ $P < 0.0001$	$r = 0.80$ $P < 0.0001$	$r = 0.85$ $P < 0.0001$	$r = 0.90$ $P < 0.0001$
MPA-AUC_{0-2}	$r = 0.82$ $P < 0.0001$	$r = 0.86$ $P < 0.0001$	$r = 0.77$ $P < 0.0001$	$r = 0.77$ $P < 0.0001$	$r = 0.85$ $P < 0.0001$

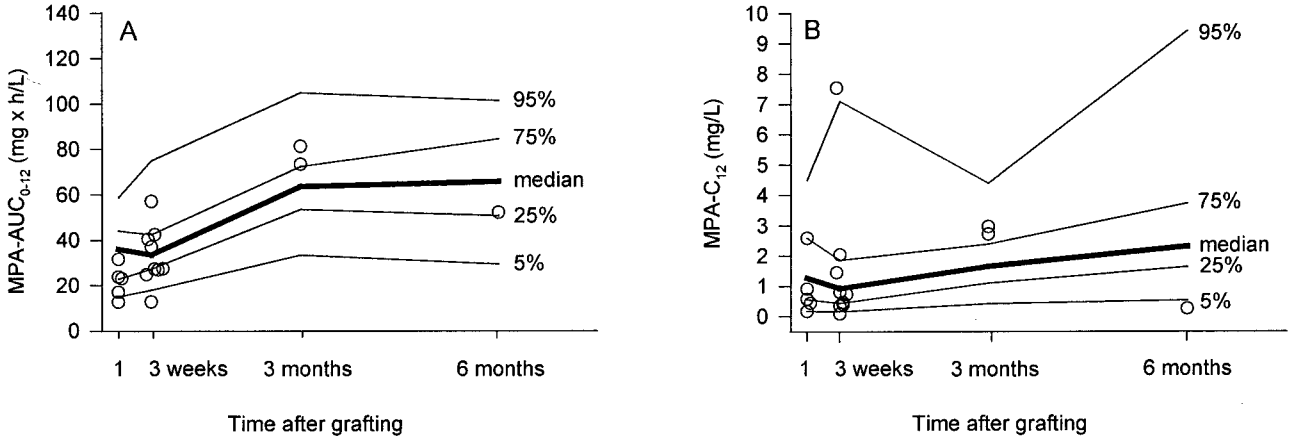

Figure 1. Time course of MPA-AUC_{0-12} (A) and predose (C_{12}) plasma levels (B) derived from HPLC measurements in the first 6 mo after grafting in pediatric renal transplant recipients; data are the median and the 5 to 95% centiles of the total patient population ($n = 54$). All patients had received the full MMF dose of 600 mg/m^2 body surface area (BSA) twice daily for at least 2 d before PK sampling. (○) Episode of an acute rejection. The respective acute rejection episodes are graphically assigned to the nearest PK sampling period preceding the clinical event. The median time period between an acute rejection episode and the preceding PK sampling period was 10 d (range, 0 to 90 d).

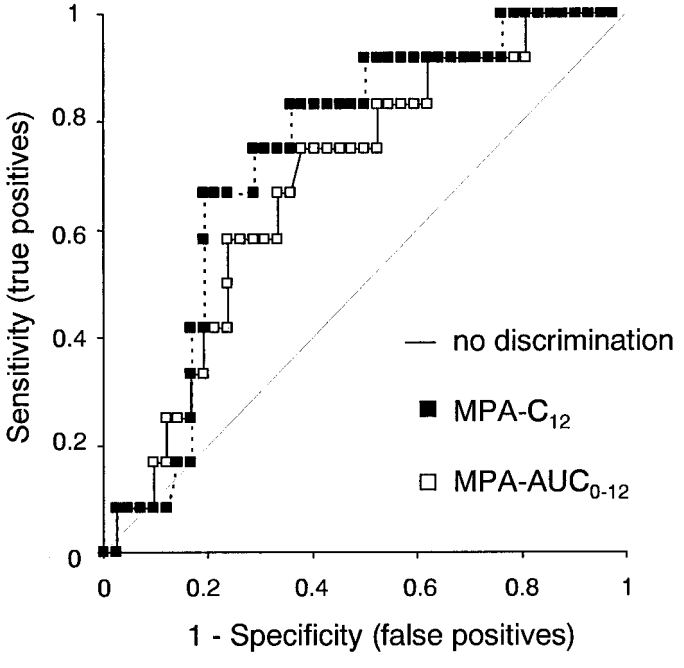

Figure 2. Receiver operating characteristic (ROC) curves illustrating the ability of the pharmacokinetic variables MPA-AUC_{0-12} (□) and predose (C_{12}) concentration (■) to discriminate between patients with ($n = 12$) and without ($n = 42$) an acute rejection episode during the first 70 d posttransplant. The broken line represents the theoretical ROC curve for no discrimination between the two groups.

Table 5. Areas under the receiver operating characteristic (ROC) curves for pharmacokinetic parameters of total and free MPA to discriminate between patients with ($n = 12$) or without an acute rejection episode during the first 70 d posttransplant

PK Parameter	Area Under the ROC Curve	95% Confidence Interval	*P*
MPA C_0 (mg/L)	0.63	0.46 to 0.79	0.07
MPA C_{12} (mg/L)	0.74	0.60 to 0.88	0.0006
MPA C_{max} (mg/L)	0.61	0.44 to 0.79	0.10
MPA-AUC_{0-12} (mg × h/L)	0.70	0.55 to 0.86	0.005
MPA-$AUC_{0,75min,4h}$ (mg × h/L)	0.70	0.53 to 0.87	0.009
MPA-AUC_{0-2} (mg × h/L)	0.65	0.49 to 0.81	0.03
Free MPA C_0 (mg/L)	0.53	0.36 to 0.71	0.36
Free MPA C_{12} (mg/L)	0.63	0.43 to 0.84	0.10
Free MPA C_{max} (mg/L)	0.51	0.30 to 0.71	0.47
Free MPA-AUC_{0-12} (mg × h/L)	0.52	0.33 to 0.72	0.40

were distributed in the upper PK centiles (Figure 3). The ROC plot of sensitivity *versus* 1-specificity for the PK variables free MPA-AUC_{0-12} and $-C_{max}$ is shown in Figure 4, and the statistical comparison of the areas under the ROC curves is given in Table 6 (initial phase) and Table 7 (stable phase posttransplant). Free MPA-AUC_{0-12} and free MPA-C_{max} were better able to discriminate patients with infections and/or leukopenia from those without these complications. The decision level in the initial phase after renal transplantation above which there is an enhanced risk of leukopenia and/or infections in the initial phase posttransplant was a free MPA-AUC_{0-12} of 0.4 mg × h/L with a diagnostic sensitivity of 92.3% and a diagnostic specificity of 61.0%. In contrast, PK variables of total MPA were discriminatory for these side effects in neither the initial (Table 6) nor the stable phase posttransplant (Table 7). There was also no association between the incidence of diarrhea or thrombocytopenia and any of the PK parameters derived from measurements of total or free MPA (results not shown).

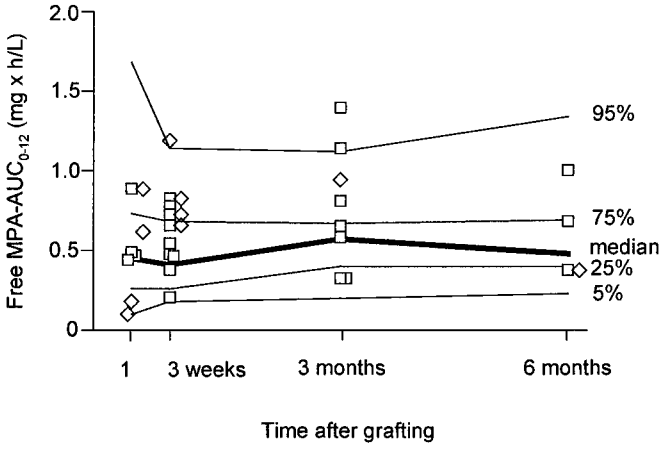

Figure 3. Time course of free MPA-AUC_{0-12} values in the first 6 mo after grafting in pediatric renal transplant recipients; data are the median and the 5 to 95% centiles of the total patient population (n = 54). All patients had received the full MMF dose of 600 mg/m^2 BSA twice daily for at least 2 d before PK sampling. Moderate (□) or severe (◇) adverse events of leukopenia and/or infection are indicated accordingly. The respective adverse events are graphically assigned to the nearest PK sampling period preceding the event. The median time period between an adverse event and the preceding PK sampling period was 14 d (range, 0 to 38 d).

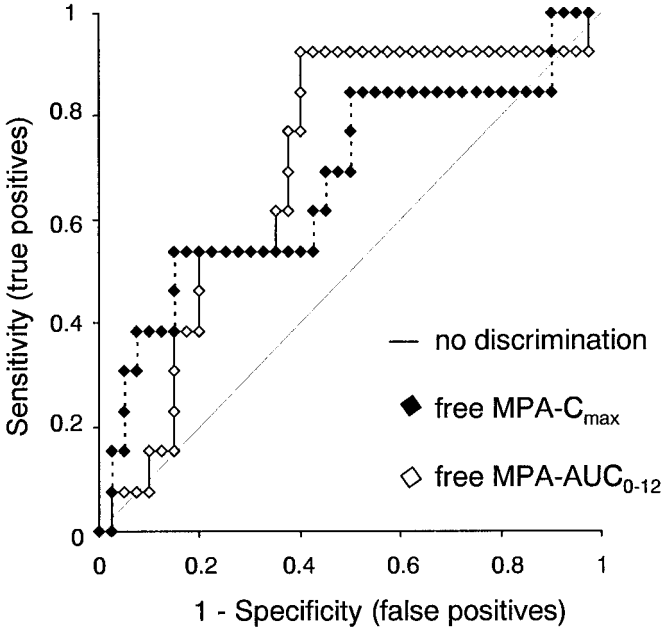

Figure 4. Receiver operating characteristic curves illustrating the ability of the pharmacokinetic variables free MPA-AUC_{0-12} (◇) and C_{max} concentration (◆) to discriminate between patients with (n = 13) and without (n = 41) adverse events of leukopenia (n = 3) and/or infection (n = 10) during the first 70 d posttransplant. The broken line represents the theoretical ROC curve for no discrimination between the two groups.

Table 6. Areas under the ROC curves for pharmacokinetic parameters of total and free MPA to discriminate between patients with or without adverse events of infections (n = 18) and/or leukopenia (n = 2) during the first 70 d posttransplant

PK Parameter	Area Under the ROC Curve	95% Confidence Interval	P
MPA C_0 (mg/L)	0.59	0.42 to 0.75	0.15
MPA C_{12} (mg/L)	0.64	0.48 to 0.81	0.06
MPA C_{max} (mg/L)	0.51	0.29 to 0.72	0.48
MPA-AUC_{0-12} (mg × h/L)	0.58	0.40 to 0.75	0.20
MPA-$AUC_{0,75min,4h}$ (mg × h/L)	0.51	0.33 to 0.68	0.48
MPA-AUC_{0-2} (mg × h/L)	0.58	0.40 to 0.77	0.18
Free MPA C_0 (mg/L)	0.56	0.37 to 0.74	0.29
Free MPA C_{12} (mg/L)	0.52	0.35 to 0.69	0.41
Free MPA C_{max} (mg/L)	0.68	0.50 to 0.86	0.03
Free MPA-AUC_{0-12} (mg × h/L)	0.71	0.55 to 0.87	0.005

Table 7. Areas under the ROC curves for pharmacokinetic parameters of total and free MPA to discriminate between patients with or without adverse events of infections (n = 9) and/or leukopenia (n = 3) in the stable phase posttransplant

PK Parameter	Area Under the ROC Curve	95% Confidence Interval	P
MPA C_0 (mg/L)	0.51	0.32 to 0.70	0.46
MPA C_{12} (mg/L)	0.53	0.33 to 0.73	0.38
MPA C_{max} (mg/L)	0.59	0.40 to 0.78	0.18
MPA-AUC_{0-12} (mg × h/L)	0.56	0.37 to 0.76	0.26
MPA-$AUC_{0,75min,4h}$ (mg × h/L)	0.63	0.44 to 0.82	0.09
MPA-AUC_{0-2} (mg × h/L)	0.52	0.34 to 0.71	0.41
Free MPA C_0 (mg/L)	0.56	0.36 to 0.76	0.28
Free MPA C_{12} (mg/L)	0.55	0.34 to 0.76	0.32
Free MPA C_{max} (mg/L)	0.71	0.55 to 0.87	0.006
Free MPA-AUC_{0-12} (mg × h/L)	0.71	0.54 to 0.88	0.007

Discussion

This is the first study that investigated the PK/PD relationship for MMF in immunosuppressive therapy in pediatric renal transplant recipients and the first that establishes the role for measurement of free MPA for the assessment of the toxic risk of MMF therapy. We have shown that both MPA-AUC_{0-12} and predose MPA levels are significantly associated with the risk of acute rejection in this patient population. By ROC analysis, an AUC_{0-12} of 33.8 mg × h/L in the initial phase posttransplant had a diagnostic sensitivity of 75% and a diagnostic specificity of 64% for discrimination of patients with acute rejections. The respective discrimination threshold for the

MPA predose concentration was 1.2 mg/L with a sensitivity of 83% and a specificity of 64%.

The incidence of the MMF-related adverse events, diarrhea, vomiting, or abdominal pain, was not associated with MPA PK variables, which is consistent with previous findings in adult renal transplant recipients (10). These adverse events might also reflect local effects of MMF in the gastrointestinal system and not only systemic exposure. The adverse events, leukopenia and/or infections, were also not associated with any of the PK variables derived from total MPA measurements but with the AUC_{0-12} of free MPA. Most of these adverse events were associated with free MPA-AUC_{0-12} levels >0.4 mg $\times$ h/L (Figure 3). MPA is extensively bound to albumin, with a range of protein binding of 97% to 99% in patients with normal renal and liver function (3–5). Factors that alter protein binding can affect the relationship between total concentration and free concentration. The pharmacologically inactive phenolic glucuronide metabolite of MPA, MPAG, competes for the albumin binding sites and can increase the fraction of unbound active MPA when present at high concentrations, for example as a consequence of renal failure (5,19). The presence of hyperbilirubinemia, uremia, low pH, and low albumin concentrations also can decrease MPA binding (3–5,19). For this reason, under circumstances of perturbed binding, the interpretation of total MPA plasma concentrations must take into account the altered MPA binding. This observation of an increased toxic risk of MMF therapy in patients with hypoalbuminemia and/or reduced renal function is clinically important, because MMF is increasingly used for immunosuppressive therapy in patients with chronic glomerulonephritis and the nephrotic syndrome (22). In this context, it is of interest to note that Kaplan *et al.* (23) reported a case of severe leukopenia that was associated with renal insufficiency in a pancreas/renal transplant recipient receiving 0.75 g of MMF twice daily. In this case, the free MPA-AUC_{0-12} was substantially elevated (5.1 mg $\times$ h/L) and the total MPA-AUC_{0-12} was in the range observed for stable renal transplant recipients.

The apparent discrepancy that the AUC_{0-12} of total MPA was associated with the incidence of acute rejection episodes, whereas the AUC_{0-12} of free MPA was associated with side effects reflecting the systemic exposure to MMF deserves comment. It is possible that a low protein binding of MPA favors its access from the circulation to the bone marrow, where it induces leukopenia by inhibition of leukocyte maturation from precursor cells, whereas inhibition of proliferation of circulating lymphocytes is mainly responsible for its immunosuppressive effect. A recent preliminary report in stable kidney transplant recipients has confirmed the relation between free MPA levels and the incidence of leukopenia (24).

In agreement with our previous reports in smaller cohorts of patients (5,11), there was a large interindividual variation of PK data (Table 2). In the first week posttransplant, but not at later PK sampling periods, low MPA-AUC_{0-12} values were associated with young age, low serum albumin, and decreased transplant function. This transient association between impaired renal function and low MPA-AUC_{0-12} values in the initial but not stable phase posttransplant has recently been reported in adult renal transplant recipients (25). Patients with decreased renal function have an increased MPA free fraction because the accumulation of the renal metabolite MPAG competes with MPA for binding sites on albumin (5,26,27). Hence, the most likely explanation for the association between low MPA-AUC_{0-12} values and impaired renal function is that decreased protein binding in these patients leads to an enhanced MPA metabolism, because it is the free drug that is primarily available for metabolism and excretion. Other possible causes of the interindividual variability of MPA-AUC_{0-12} values include differences in the absorption of MMF and/or in the metabolism via the glucuronidation pathway.

Our study has demonstrated a significant relationship between both MPA-AUC_{0-12} and predose MPA levels and the risk of acute rejection. This observation provides some encouragement that therapeutic drug monitoring with individualized dosing of MMF could optimize the usage of this immunosuppressive drug. There are additional lines of evidence that favor such a monitoring. First, MPA displays highly variable interindividual pharmacokinetics; in the initial phase posttransplant, there is also considerable intraindividual PK variability (3,5,8,11). Second, there is a time-dependent increase in MPA-AUC_{0-12} in both adult and pediatric renal transplant recipients on concomitant immunosuppression with cyclosporin A and corticosteroids (8,11) that might require time-dependent dose adjustments of MMF. Third, the pharmacokinetics of MPA is also dependent on the concomitant immunosuppression (28). However, it has to be emphasized that the value of therapeutic drug monitoring of MPA has not been formally tested yet. Only a prospective trial in which kidney transplant recipients treated with the same triple regimen are randomized for treatment with a fixed dose of MMF *versus* treatment with an MMF dose adjusted to the results of plasma concentration monitoring could establish the scientific basis of therapeutic drug monitoring of MPA. Such rigorous criteria are required to justify the additional effort and expense involved, before a general therapeutic drug monitoring of MPA can be recommended. Moreover, attempts to define the PK/PD relationship for gastrointestinal disturbances have not been successful, which is consistent with the results in this study. Instead, the dose of MMF provides the most predictive information for adverse events such as diarrhea (10), presumably because of the local toxicity of MPA on the gastrointestinal epithelium. However, given the association between MPA PK variables and the risk of acute rejection, therapeutic drug monitoring of MPA in the initial phase posttransplant could be helpful in selected high-risk patients. It would be reasonable to aim at achieving MPA-AUC values or predose MPA levels somewhat higher than those achieved in the lower percentiles by individual adjustment of the MMF dose. For such an approach, the patient-specific and immunosuppressive regimen–specific percentiles for MPA-AUC and MPA predose levels from this study could serve as a guideline for optimization of MMF therapy in pediatric renal transplant recipients. On the basis of data from this and other studies, a consensus is arising that for minimizing the risk of rejection after transplantation, total MPA-AUC values in the early stages posttransplant should be maintained

within the therapeutic window of 30 to 60 mg × h/L, based on HPLC data, or the predose concentration in the range of 1 to 3.5 mg/L. Because some fluctuations are to be expected as a consequence of the enterohepatic circulation of MPA, extreme values or a large change should be verified by repeat measurement before a change in the MMF dose is undertaken.

The question arises of which PK parameter of total and free MPA is best suited for therapeutic drug monitoring of MMF therapy in clinical practice. In this study, the parameters MPA-AUC_{0-12}, MPA-$AUC_{0,75\ min,4\ h}$, and MPA C_{12} were comparable for assessing the risk of acute rejection episodes, whereas MPA C_0 and MPA-AUC_{0-2} had a somewhat poorer predictive value (Table 5). In two previous studies in adult renal transplant recipients, the MPA-AUC was a better predictor of outcome than the predose concentration (21,29). Predose levels are more convenient than measurements of AUC that are complex and increase the cost of routine clinical monitoring. However, the predose C_{12}, *i.e.*, the concentration measured 12 h after a supervised dose, is not the predose sample commonly collected in a clinic. This is the C_0 predose concentration, which may be sampled at a more variable time interval after the last dose. Hence, the calculation of AUC using a three-point limited sampling strategy up to 4 h after MMF dosing (MPA-$AUC_{0,75\ min,4\ h}$) appears to be a more precise approach for the assessment of the risk of acute rejection than the predose C_0 level.

In conclusion, we have observed an association between the risk of acute rejection episodes and MPA-AUC values or MPA predose levels in pediatric renal transplant recipients on an immunosuppressive triple drug therapy with cyclosporin A, MMF, and corticosteroids. We have established 5^{th} to 95^{th} percentiles for these PK parameters on the basis of HPLC measurements in this specific patient population. These reference data could facilitate the therapeutic drug monitoring of MPA for optimization of MMF efficacy by steering patients away from the extreme values of MPA PK variables indicated by the lower percentiles. For the assessment of the toxic risk of MMF regarding leukopenia and/or infections, measurement of free MPA appears to be more appropriate.

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