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Pharmacokinetics of Mycophenolic Acid (MPA) and Determinants of MPA Free Fraction in Pediatric and Adult Renal Transplant Recipients

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Abstract. Dosage guidelines for mycophenolate mofetil (MMF), an ester prodrug of the immunosuppressant mycophenolic acid (MPA), are still preliminary in children. This study compares the pharmacokinetics of MPA and its major metabolite MPA glucuronide (MPAG) in pediatric renal transplant recipients receiving 600 mg MMF/m^2 body surface area twice a day to those of adults on the currently recommended oral dose of 1 g of MMF twice a day. Concentration–time profiles of 18 children (age, 10.7 ± 0.72 yr; range, 5.9 to 15.3 yr) and 10 adults were investigated 1 and 3 wk after transplantation. Plasma concentrations of MPA and MPAG were measured by reverse-phase HPLC. Because MPA is extensively bound to serum albumin and only the free fraction is presumed to be pharmacologically active, the MPA free fraction was also analyzed by HPLC after separation through ultrafiltration. The areas under the concentration–time curves (AUC_{0-12}) of total and free MPA throughout the 12-h dosing interval in children were, in general, comparable to the corresponding data in adult patients. The mean AUC_{0-12} of MPA and free MPA did not change significantly over the first 3 wk after transplantation, but there was substantial intra- and interindividual variation. MPAG-AUC_{0-12} values in children with primary renal transplant dysfunction were threefold higher than in those with functioning transplants. Renal impairment had no consistent effect on total MPA-AUC_{0-12} values, but the MPA free fraction in children (median, 1.65%; range, 0.40 to 13.8%) was significantly (r^2 = 0.46) modulated by renal transplant function and serum albumin levels. In conclusion, concentration–time profiles of pediatric renal transplant recipients administered 600 mg MMF/m^2 body surface area twice a day are comparable to those in adults on 1 g MMF twice a day in the first 3 wk after transplantation. Renal impairment and decreased serum albumin levels led to an increase in the free fraction of MPA and the free MPA-AUC_{0-12} values. Because the pharmacologic activity of MPA is a function of unbound drug concentration, these findings might be relevant for the pharmacodynamic effects of MPA.

Mycophenolate mofetil (MMF), an ester prodrug of the immunosuppressant mycophenolic acid (MPA), has recently been approved both in adults and children for maintenance immunosuppressive therapy after renal transplantation. MPA is a potent, reversible, noncompetitive inhibitor of inosine monophosphate dehydrogenase, and thus MPA acts as a relatively selective inhibitor of T and B cell proliferation by blocking the production of guanosine nucleotides and interfering with the glycosylation of adhesion molecules (1–3). In three randomized, double-blind multicenter clinical trials, patients treated with MMF in conjunction with cyclosporine and corticosteroids showed reduced incidence and severity of rejection episodes, similar graft survival, and better graft function over 12 mo *versus* azathioprine-treated patients (4,5) or placebo (6). However, there is only limited clinical experience with MMF in pediatric renal transplant recipients (7,8). In particular, no detailed analysis of the pharmacokinetics of MPA in children has been published, and dosage guidelines are still preliminary. One purpose of this study was to evaluate the pharmacokinetics of MPA in pediatric transplant recipients in the first weeks after renal transplantation compared with adult patients receiving the currently recommended dose of 1000 mg of MMF twice a day.

After oral administration, MMF is rapidly and essentially

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completely absorbed, and then essentially completely converted to MPA, the active immunosuppressant species. A secondary plasma MPA peak is often seen 6 to 12 h after oral administration of MMF, suggesting enterohepatic circulation of the drug (9). Because of this secondary rise in plasma MPA concentration, the apparent mean terminal half-life of MPA is 17.9 h in healthy subjects (10). MPA is converted in the liver to the pharmacologically inactive MPA glucuronide (MPAG), which is excreted by the kidney (9). Plasma MPA is extensively bound to albumin, and a mean protein binding of 97% has been reported in normal plasma (9). *In vitro* studies have suggested that the free MPA concentration may more accurately reflect the degree of immunosuppressive action of the drug (*i.e.*, inhibition of inosine monophosphate dehydrogenase and the proliferative response of mononuclear cells to mitogenic stimulation) than does the concentration of total MPA (11). Previous pharmacokinetic investigations of MPA measured only the plasma concentration of total MPA, which is the sum of bound and free MPA. However, changes of plasma protein binding might lead to considerable alterations of free MPA concentration *in vivo* without corresponding changes of the total MPA concentration. Therefore, we have also analyzed free MPA plasma concentrations and evaluated factors that might modulate the MPA free fraction in renal transplant recipients.

Materials and Methods

Patients

This study was an open-label evaluation of the pharmacokinetics of MPA in pediatric renal transplant recipients. Inclusion criteria were: (*1*) patients who had received a first or second cadaveric or living-related donor kidney transplant and who were eligible to receive oral medication on or before day 3 after transplantation; and (*2*) patient age ≤18 yr. Exclusion criteria were: (*1*) patients who received any immunosuppressive therapy other than a standard prophylactic regimen with cyclosporin A, corticosteroids, and MMF; (*2*) patients with severe gastrointestinal disorders that interfered with their ability to receive or absorb oral medication and patients with severe diarrhea; (*3*) patients or their donors with serologic evidence of human T cell leukemia virus, HIV, or hepatitis B or C; (*4*) patients with malignancies or history of malignancy; (*5*) patients with systemic infections requiring therapy at the time of entry into the study; (*6*) patients with a white blood cell count ≤2500/μl or hemoglobin concentration ≤5 g/dl; or (*7*) concomitant medication with the following drugs: azathioprine, rapamycin, desoxyspergualin, cyclophosphamide, methotrexate, vincristine, prostaglandin E1 or E2, brequinar sodium, mizoribin, aciclovir, or probenecid. No patient received cholestyramine, magnesium- or aluminum hydroxide-containing antacids or aspirin.

Characteristics of 18 pediatric patients who were entered into the study are listed in Table 1. Thirteen of them had primary transplant function, and five patients had delayed graft function defined as the requirement for dialysis in the first 3 posttransplant weeks. Immunosuppressive therapy consisted of 300 mg methylprednisolone/m^2 body surface area (BSA) on the day of transplant surgery, followed by 60 mg/m^2 for the first week, 30 mg/m^2 for the second week, and 15 mg/m^2 for the third week after transplantation. Cyclosporin A (microemulsion formulation) was administered in a dose of 500 mg/m^2 per d given in two divided doses for 24 h, starting 6 h after surgery. Thereafter, doses (approximately 300 mg/m^2 BSA per d) were ad-

Table 1. Patient characteristics[a]

Group	Gender (M/F)	Age (yr)	Weight (kg)	BSA (m^2)	Primary TPL Function	GFR (ml/min per 1.73 m^2)		Serum Albumin (g/L)	
						1 wk	3 wk	1 wk	3 wk
Children (n = 18)	10/8	10.7 ± 0.72 (5.9 to 15.3)	29.3 ± 2.49 (16.0 to 50.3)	1.02 ± 0.06 (0.68 to 1.50)	13/18	57.0 ± 9.07 (7.0 to 133)	88.4 ± 6.75 (46.3 to 143)	36.0 ± 1.74 (24.0 to 51.0)	37.6 ± 1.02 (27.0 to 47.0)
Adults (n = 10)	8/2	45.9 ± 4.1 (20.1 to 59.2)	78.7 ± 3.2 (65.8 to 98.4)	1.93 ± 0.04 (1.79 to 2.18)	5/10	27.1 ± 8.0 (7.0 to 77.8)	50.3 ± 7.4 (7.0 to 86.5)	35.1 ± 1.3 (29.0 to 40.0)	40.0 ± 1.32 (32.0 to 47.0)

[a] Data are mean ± SEM (range). BSA, body surface area; TPL, transplant.

justed 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).

A group of 10 adult renal transplant recipients was concomitantly studied for comparison by use of the same pharmacokinetic protocol. Patient characteristics are listed in Table 1. With the exception of patient age, the same inclusion and exclusion criteria were applied as for the pediatric patients. No graft loss occurred in either patient group.

MMF Dosage

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 (12). 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. Adult patients received the currently recommended oral dose of 1 g of MMF twice a day in capsules. BSA was calculated by the formula of Du Bois and Du Bois (13).

Pharmacokinetic Protocol

Patients were studied after informed (parental) consent was obtained. Blood samples for pharmacokinetic assessment were obtained on days 7 (early postoperative period) and 21 (late postoperative period) after renal transplantation. It was mandatory that all patients had at least 2 full days of the same MMF dose given twice a day before pharmacokinetic investigation. The study was performed under in-patient 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 as follows: 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 ethylenediaminetetra-acetic 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

MPA, MPAG, and the carboxy butoxy ether of MPA were kind gifts of Hoffmann-La Roche (Grenzach-Wyhlen, Germany). Plasma concentrations of MPA and MPAG were determined by reverse-phase HPLC, using a Symmetry-C18 column (Waters Associates, Milford, MA). Briefly, 200 μl of ethylenediaminetetra-acetic acid plasma was mixed with 100 μl of acetonitrile containing the carboxy butoxy ether of MPA (15 mg/L) as internal standard. This was followed by sequential addition of 20 μl of perchloric acid (150 g/L) and 20 μl of sodium tungstate solution (250 g/L). After mixing and centrifugation, 50 μl of supernatant was applied to the C-18 column. The mobile phase for elution of the column consisted of solution A (250 ml of acetonitrile and 750 ml of 20 mM phosphate buffer, pH 3.0). and solution B (700 ml of acetonitrile and 300 ml of 20 mM phosphate buffer, pH 6.5), which formed the following gradient: 0 to 4.5 min 3% B; 5 to 12 min 30% B; 12.5 to 14.5 min 100% B. Compounds were quantified in parallel by absorbance at 254 and 215 nm. For calibration of MPA and MPAG, drug-free plasma was spiked with either of the two compounds at concentrations of 3 and 200 mg/L, respectively. Using drug-free plasma spiked with MPA or MPAG, the method was found to be linear up to 50 mg/L for MPA and 500 mg/L for MPAG. The detection limit (signal-to-noise ratio of 3) at 215 nm for plasma samples was 0.01 mg/L for total MPA and 0.03 mg/L for MPAG. Between-run imprecision ranged from 3.3 to 9.2% for MPA and 4.1 to 6.1% for MPAG.

The Centrifree Micropartition System (Amicon, Beverly, MA) as described by Nowak and Shaw (11) was used to obtain an ultrafiltrate for free MPA determination. Because some batches of filters were found to contain impurities that interfered with the chromatography, all filters were routinely washed with methanol water (1:1 dilution, vol/vol) before use. For the ultrafiltration procedure, 300 μl of plasma was added to the sample reservoir and the tube was centrifuged at 2000 × *g* (20°C) for 40 min, yielding approximately 150 μl of ultrafiltrate. This was mixed with internal standard (2.5 mg/L) at a ratio of 10:1 (vol/vol), and 100 μl was then injected directly into the C-18 column. A solution of 9 g/L NaCl adjusted to pH 7.4 with phosphate buffer (67 mmol/L) and spiked with 0.05 mg/L MPA was used for calibration of free MPA determination. The detection limit for free MPA at 215 nm was 0.005 mg/L. Because of an imprecision of >20% at 0.005 mg/L, the limit of quantification for free MPA was set at 0.01 mg/L. The within-day imprecision ranged from 6.5 to 11.8% and the between-day imprecision from 7.2 to 15.8%. Before starting this investigation, it was confirmed that freezing and thawing of samples did not influence the protein binding of MPA.

Serum Biochemistry

Serum albumin and serum and urinary creatinine were measured by standard automated procedures. In children, GFR was estimated using the formula of Schwartz and stratified according to the age and gender of the patients (14). In adult patients, creatinine clearance was calculated from a 24-h urine collection using the standard formula. In patients with primary transplant dysfunction, no attempt was made to measure residual GFR, which is usually in the very low range, between 5 and 10 ml/min per 1.73 m^2. Therefore, a value of 7 ml/min × 1.73 m^2 was arbitrarily entered.

Pharmacokinetic Analysis

The following pharmacokinetic data for MPA, free MPA, and MPAG were determined: 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 minimum concentration (C_{min} [mg/L]). Because $C_{time\ 0}$ and $C_{time\ 12}$ values for MPA, free MPA, and MPAG were not significantly different in children or adults, C_{min} was defined by the formula: $C_{min} = (C_{time\ 0} + C_{time\ 12})/2$. The pharmacokinetic analysis was performed using the computer program BiAS (Epsilon-Verlag Hochheim, Darmstadt, Germany).

Statistical Analyses

The Shapiro–Wilks test was used to confirm normality of data (15). Because some parameters were normally distributed and some were not, data in Tables 2 and 3 are given as means ± SEM and as median (range) to allow a better comparison of the data. For comparison between two normally distributed groups, an unpaired or paired *t* test (two-tailed) was used, as appropriate. For comparison between two non-normally distributed groups, the Mann–Whitney test or Wilcoxon signed rank test was used, as appropriate. Correlations between variables were assessed using univariate linear regression analysis. Forward stepwise regression analysis was used to identify independent predictors of the MPA free fraction. Differences were considered statistically significant at $P < 0.05$.

Table 2. Pharmacokinetic parameters of MPA, free MPA, and MPAG in pediatric renal transplant recipients[a]

Parameter	T_{max} (h)		C_{max} (mg/L)		C_{min} (mg/L)		AUC_{0-12} (mg × h/L)	
	1 wk	3 wk	1 wk	3 wk	1 wk	3 wk	1 wk	3 wk
MPA (n = 18)	1.56 ± 0.37[b]	1.0 ± 0.13[b,c]	12.5 ± 1.47[b,d]	19.6 ± 2.51[e]	1.48 ± 0.24[b,c]	1.05 ± 0.21[b]	36.5 ± 2.89[d]	34.0 ± 3.09
	0.66	1.25	9.36	18.8	1.04	0.81	40.0	33.5
	(0.33 to 6.0)	(0.33 to 2.0)	(5.01 to 25.9)	(5.28 to 45.7)	(0.27 to 3.98)	(0.17 to 3.9)	(19.2 to 55.6)	(13.9 to 58.4)
Free MPA (n = 18)	1.59 ± 0.36[b]	1.12 ± 0.21[b]	0.23 ± 0.02	0.30 ± 0.04	0.03 ± 0.01[b]	0.01 ± 0.002	0.70 ± 0.12	0.50 ± 0.06
	1.25	0.96	0.23	0.26	0.02	0.01	0.52	0.46
	(0.33 to 6.0)	(0.33 to 4.0)	(0.06 to 0.39)	(0.09 to 0.65)	(<0.01 to 0.12)	(<0.01 to 0.03)	(0.24 to 2.30)	(0.14 to 1.16)
MPAG (n = 18)	3.06 ± 0.47[b]	2.11 ± 0.26[b]	179 ± 28.5[b]	129 ± 9.13[d]	112 ± 28.2[b,c]	53.1 ± 7.2[f,g]	1654 ± 337[b]	987 ± 92.8[f,d]
	2.0	2.0	129	120	67.3	49.3	1201	968
	(1.25 to 8.0)	(1.25 to 4.0)	(84.0 to 511)	(73.0 to 229)	(17.0 to 345)	(11.0 to 126)	(436 to 5531)	(410 to 1780)

[a] Data are mean ± SEM and median (range). MPA, mycophenolic acid; MPAG, MPA glucuronide; T_{max}, time to maximum concentration; C_{max}, maximum concentration; C_{min}, minimum concentration; AUC_{0-12}, area under the curve from 0 to 12 h.
[b] Normality test failed.
[c] $P < 0.05$, children *versus* adults.
[d] $P < 0.01$, children *versus* adults.
[e] $P < 0.05$, 3 wk *versus* 1 wk.
[f] $P < 0.01$, 3 wk *versus* 1 wk.
[g] $P < 0.001$, children *versus* adults.

Table 3. Pharmacokinetic parameters of MPA, free MPA, and MPAG in adult renal transplant recipients[a]

Parameter	T_{max} (h)		C_{max} (mg/L)		C_{min} (mg/L)		AUC_{0-12} (mg × h/L)	
	1 wk	3 wk	1 wk	3 wk	1 wk	3 wk	1 wk	3 wk
MPA (n = 10)	1.85 ± 0.72[b]	1.51 ± 0.18[b]	7.03 ± 1.33	10.2 ± 1.92	0.73 ± 0.12	1.22 ± 0.42[b]	22.1 ± 1.78	30.9 ± 4.55[b]
	1.25	1.63	5.98	8.87	0.73	0.82	20.5	27.5
	(0.33 to 8.0)	(0.66 to 2.0)	(2.40 to 15.7)	(2.59 to 24.7)	(0.14 to 1.27)	(0.19 to 4.79)	(16.5 to 36.1)	(14.4 to 58.8)
Free MPA (n = 10)	2.6 ± 0.83[b]	1.37 ± 0.19	0.21 ± 0.03	0.21 ± 0.03	0.02 ± 0.01	0.02 ± 0.005	0.80 ± 0.14	0.57 ± 0.05
	2.0	1.25	0.23	0.2	0.02	0.02	0.67	0.60
	(0.33 to 8.0)	(0.66 to 2.0)	(0.08 to 0.36)	(0.09 to 0.39)	(<0.01 to 0.05)	(<0.01 to 0.05)	(0.31 to 1.55)	(0.42 to 0.83)
MPAG (n = 10)	3.85 ± 1.19[b]	3.25 ± 0.77	215 ± 26.3	193 ± 22.2	163 ± 24.7	137 ± 20.6	2095 ± 259	1901 ± 212
	2.0	3.0	213	181	193	138	2288	1782
	(0 to 12.0)	(0 to 8.0)	(101 to 345)	(100 to 327)	(43.4 to 277)	(52.0 to 269)	(807 to 3017)	(943 to 2849)

[a] Data are mean ± SEM and median (range). Abbreviations as in Table 2.
[b] Normality test failed.

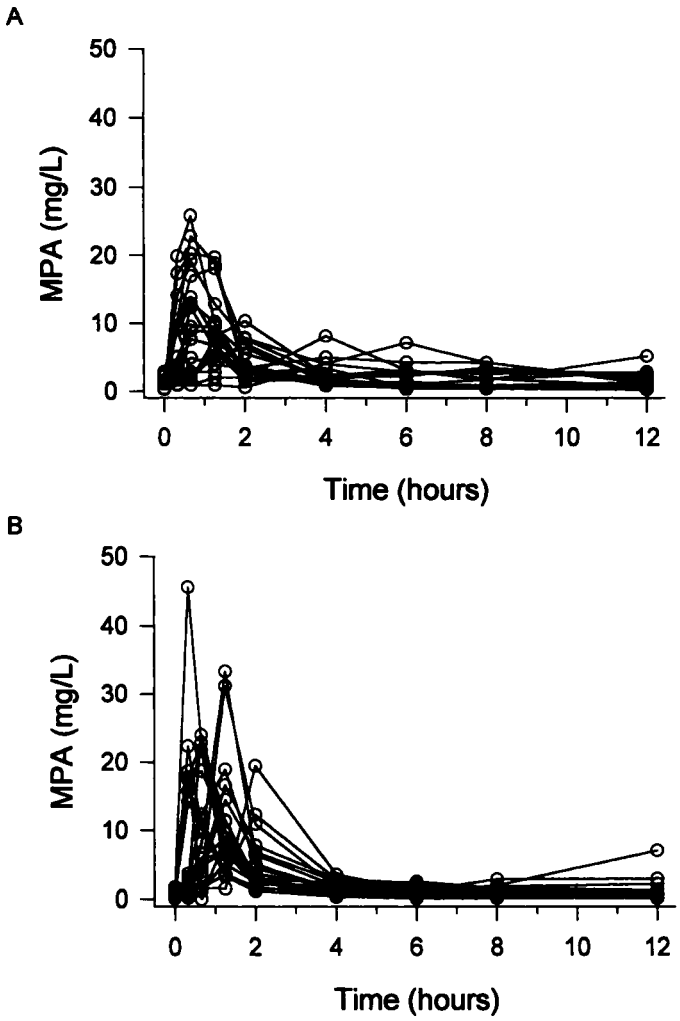

Figure 1. Individual plasma concentrations of mycophenolic acid (MPA) *versus* time (12-h dosing interval) in 18 pediatric transplant recipients after oral administration of 600 mg mycophenolate mofetil (MMF)/m^2 BSA twice a day 1 wk (A) and 3 wk (B) after renal transplantation.

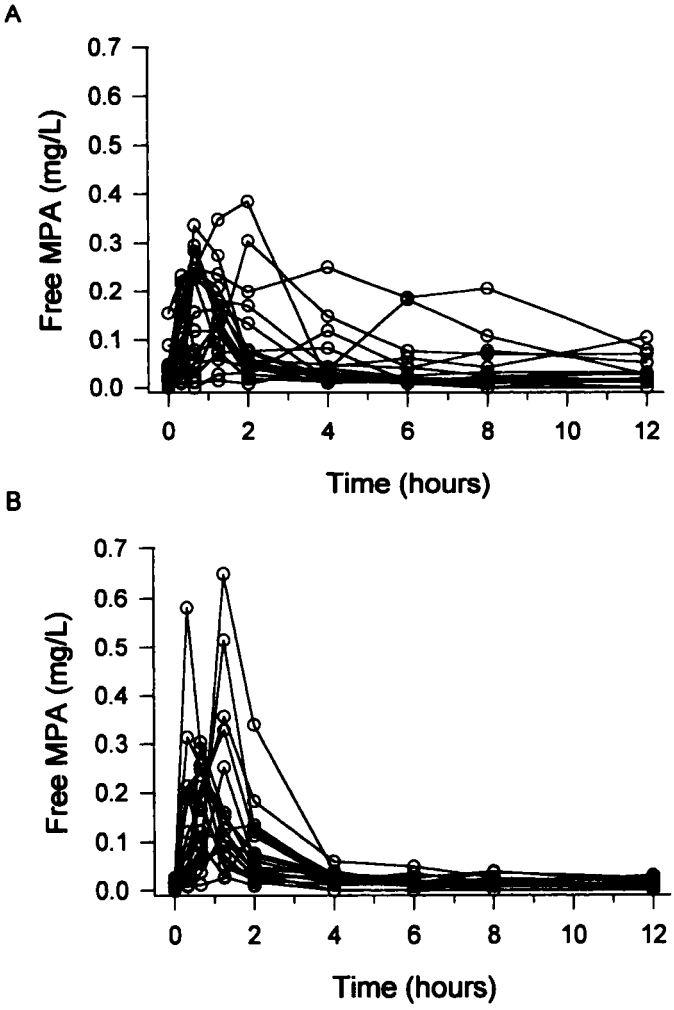

Figure 2. Individual plasma concentrations of free MPA *versus* time (12-h dosing interval) in 18 pediatric transplant recipients after oral administration of 600 mg MMF/m^2 BSA twice a day 1 wk (A) and 3 wk (B) after renal transplantation.

Results

The pharmacokinetic parameters of MPA, free MPA, and MPAG in pediatric transplant recipients are depicted in Table 2, and the corresponding data in adult patients are given in Table 3. The pharmacokinetic data in adult patients are comparable to those reported previously in the immediate post-transplant period (16). Mean MPA-AUC_{0-12} in pediatric patients 1 wk after renal transplantation was 40% higher than in adults, but comparable at 3 wk, when a functioning renal transplant was observed in all but one patient. The AUC_{0-12} values of free MPA at 1 and 3 wk did not differ between children and adults (Tables 2 and 3). The higher AUC_{0-12} values for the MPA metabolite MPAG in adult patients compared with children during both pharmacokinetic investigations are most likely due to the higher incidence of primary transplant dysfunction in the former group (Table 1). MPAG-AUC_{0-12} values at 1 wk in children with primary renal transplant dysfunction (3321 ± 850 mg × h/L; $n = 5$) were threefold higher than in those with functioning transplants (1013 ± 83 mg × h/L; $n = 13$; $P < 0.001$). There was a tight inverse correlation between the plasma MPAG-AUC_{0-12} values and GFR both in children ($r = -0.70$, $P < 0.001$) and adults ($r = -0.83$, $P < 0.001$).

Individual MPA plasma concentrations in 18 children after 1 and 3 wk of MMF treatment are depicted in Figure 1. The plasma profiles of MPA after oral administration of MMF showed an early and sharp increase. The peak concentration (T_{max}) of MPA was reached at 45 to 75 min after dosing. These profiles are consistent with rapid absorption and rapid conversion of MMF to MPA, followed by rapid distribution and metabolism of the generated MPA. Small secondary increases in plasma MPA levels occurred in individual patients 4 to 12 h after administration, consistent with the previously described enterohepatic circulation of MPAG, which undergoes deglucuronidation and reabsorption as MPA (17). Because these increases interfered with accurate calculation of terminal half-life of MPA, we do not report half-life values.

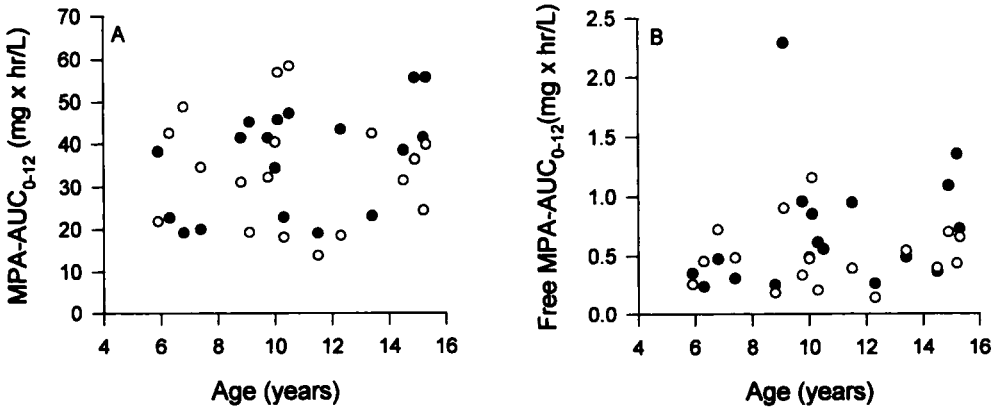

Figure 3. Areas under the concentration–time curve (AUC_{0-12}) values for MPA (A) and free MPA (B) 1 wk (●) and 3 wk (○) after renal transplantation in 18 pediatric transplant recipients as a function of patient age.

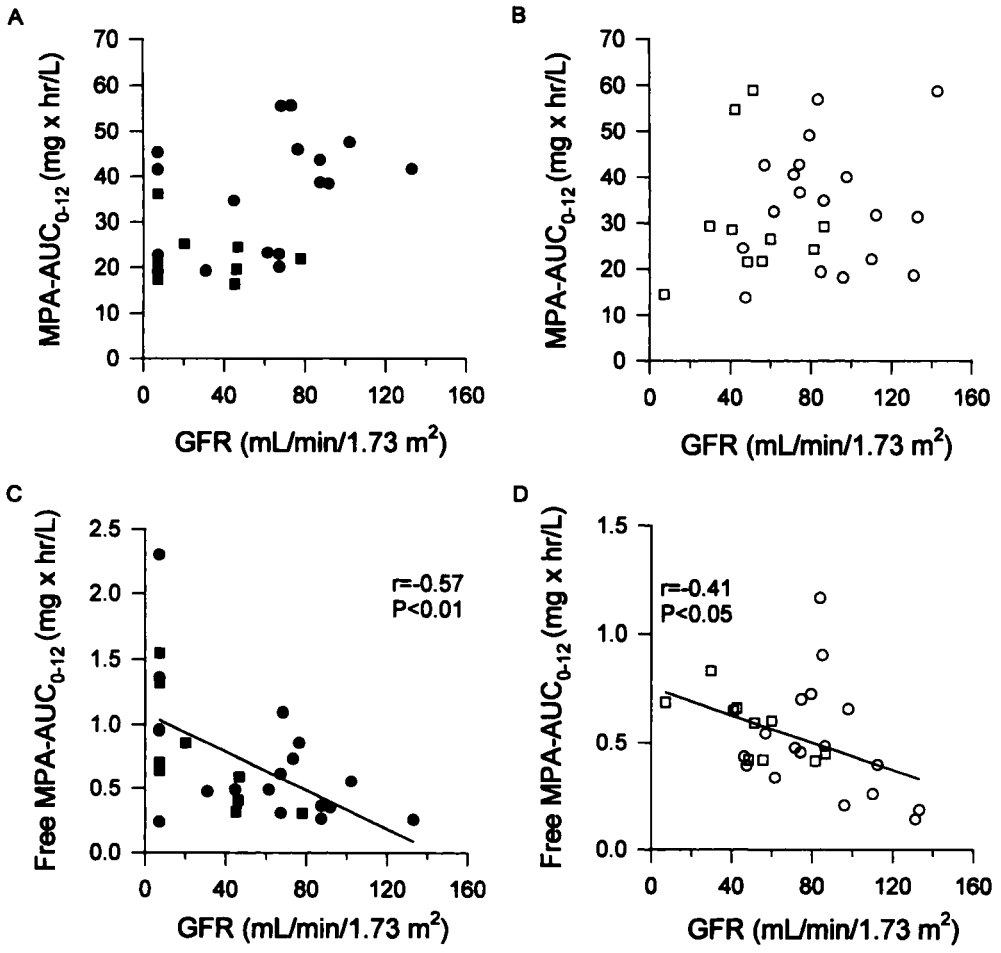

Figure 4. AUC_{0-12} values for MPA (A and B) and free MPA (C and D) in pediatric (circles) and adult (squares) renal transplant recipients 1 wk (closed symbols) and 3 wk (open symbols) after renal transplantation as a function of GFR, as estimated by creatinine clearance. Free, but not total, MPA-AUC_{0-12} values were inversely correlated with GFR at both times of investigation.

The median C_{max} at the 3-wk profile in children was twofold higher than that at the 1-wk profile, suggesting slower MMF absorption and/or conversion to MPA in the immediate postoperative period. To determine whether this difference was due to the general disease state postsurgery or to persisting uremia, pharmocokinetic parameters of pediatric and adult patients at 1 wk were stratified according to the function of the kidney transplant. Patients with primary transplant dysfunction (n = 10) had a significantly lower maximal MPA concentration at 1 wk (median C_{max}, 6.5 mg/L; range, 2.4 to 13 mg/L) and a later

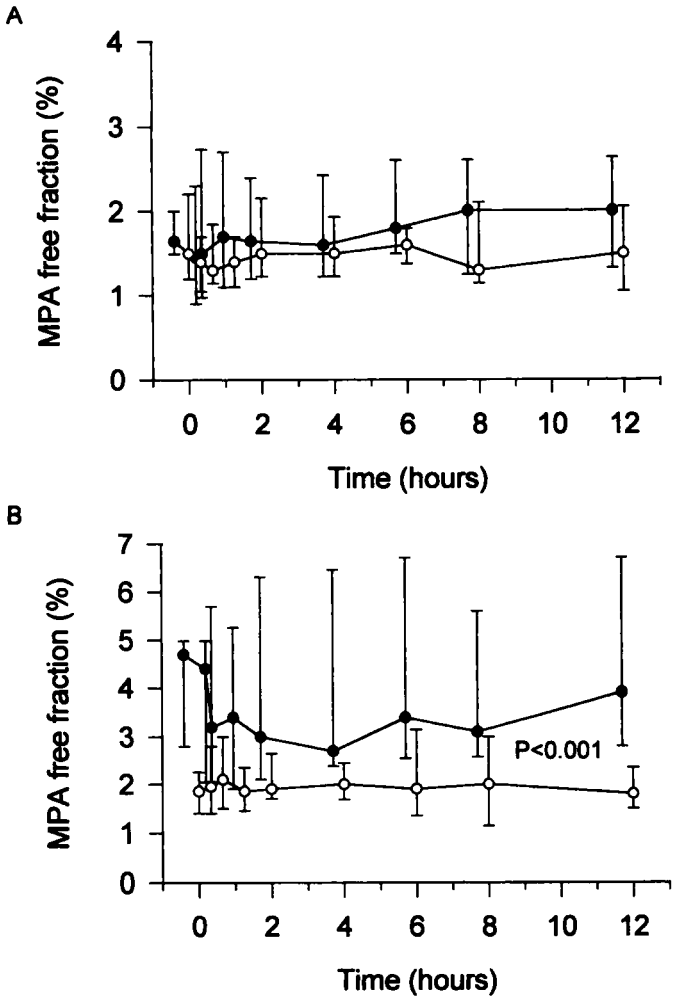

Figure 5. MPA free fraction in pediatric (A) and adult (B) renal transplant recipients at 1 wk (●) and 3 wk (○) after renal transplantation *versus* time (12-h dosing interval). Data are given as median and as the 25th and 75th percentile. In adult patients, the MPA free fraction analyzed over all time points was significantly ($P < 0.001$) lower at 3 wk *versus* 1 wk.

T_{max} (median, 2.0 h; range, 0.66 to 8.0 h) than those with primary functioning transplants ($n = 18$) (C_{max}, 12.8 mg/L; range, 3.9 to 25.9 mg/L; T_{max}, 0.66 h; range, 0.33 to 6.0 h), indicating slower absorption of MMF and/or conversion to MPA in uremic patients. Mean MPA-AUC_{0-12} did not change significantly over time, but there was a substantial interindividual variation of MPA-AUC_{0-12} values among the patients at both times of investigation (Tables 2 and 3). Moreover, there was no correlation between MPA-AUC_{0-12} values at 1 and 3 wk both in children ($r = 0.13$, $P = 0.61$) and in adults ($r = 0.09$, $P = 0.80$), indicating considerable intraindividual variability of the MPA concentration–time curve in the first few weeks after renal transplantation. Predose MPA levels correlated only moderately with the respective MPA-AUC_{0-12} values both in children (1 wk, $r = 0.72$, $P < 0.01$; 3 wk, $r = 0.57$, $P < 0.05$) and adults (1 wk, $r = 0.64$, $P < 0.05$; 3 wk, $r = 0.70$, $P < 0.05$).

The concentration–time profiles of free MPA displayed a similar interindividual variability to those of total MPA (Figure 2, Tables 2 and 3), but intraindividual variability was less with free MPA, as indicated by a correlation between free MPA-AUC_{0-12} values at 1 *versus* 3 wk in children ($r = 0.53$, $P < 0.05$) and adults ($r = 0.61$, $P = 0.06$). Despite this variability, the mean free MPA-AUC_{0-12} values remained constant both in children and adults (Tables 2 and 3). The scattering of the individual AUC_{0-12} values for MPA and free MPA in children was not related to the different patient age (Figure 3), indicating that an MMF dose of 600 mg/m^2 BSA results in a comparable total body drug exposure to MPA and free MPA over the age range studied. However, free but not total MPA-AUC_{0-12} values of children and adults were inversely correlated with GFR (Figure 4). Predose free MPA levels correlated only moderately with the respective free MPA-AUC_{0-12} values both in children (1 wk, $r = 0.74$, $P < 0.001$; 3 wk, $r = 0.37$, $P = 0.19$) and adults (1 wk, $r = 0.84$, $P < 0.01$; 3 wk, $r = 0.71$, $P < 0.05$).

Because MPA is extensively bound to serum albumin and only the free concentration of MPA in plasma water is presumed to be pharmacologically active (11), we investigated further the relation between total and free MPA at different plasma concentrations at 1 and 3 wk after renal transplantation. In pediatric patients, the MPA free fraction was similar at 1 and 3 wk (Figure 5A). There was no significant change in the free fraction of MPA throughout the 12-h dosing interval, although wide variation as indicated by the large data range was observed (Figure 5A). In the adult group, the MPA free fraction was 65% higher at 1 wk *versus* 3 wk after renal transplantation (Figure 5B). This difference is most likely due to the high proportion of patients with initial transplant dysfunction (see below). There was a significant linear correlation between total MPA and free MPA plasma levels both at 1 ($r = 0.66$, $P < 0.001$) and at 3 wk ($r = 0.90$, $P < 0.001$), but with a considerable variability of individual values. For example, in two different patients an MPA plasma level of 10 mg/L was related to a free MPA level of either 0.05 or 0.38 mg/L. The AUC_{0-12} values of total and free MPA did not correlate at 1 wk and correlated only moderately at 3 wk after transplantation (Figure 6).

Next, we sought to identify possible determinants of the MPA free fraction *in vivo*. In the pediatric group, the MPA free fraction was inversely correlated with serum albumin levels (Figure 7A) and GFR (Figure 7B) and positively correlated with MPAG-AUC_{0-12} values ($r = 0.47$, $P < 0.01$), but not with hemoglobin levels. There was also a significant correlation between serum albumin levels and GFR ($r = 0.53$, $P < 0.005$). Forward stepwise regression analysis showed that the free fraction of MPA is significantly related to serum albumin and GFR ($r^2 = 0.46$). In the adult group, the MPA free fraction was also inversely correlated with GFR ($r = -0.70$, $P < 0.001$) and MPAG-AUC_{0-12} values ($r = -0.64$, $P < 0.005$). The lack of correlation between the MPA free fraction and serum albumin in adult patients is most likely due to the smaller number of individuals investigated and the narrower range of albumin values compared with the pediatric group (Table 1). It is noteworthy that patients with a higher MPA free

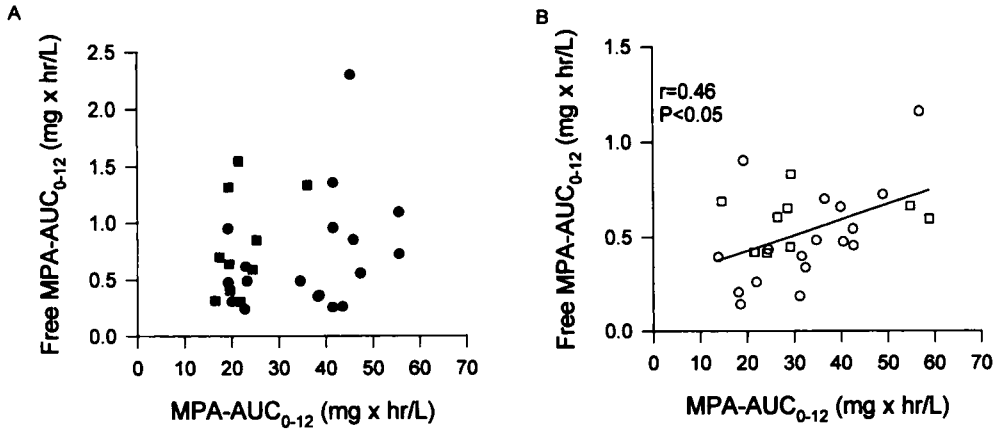

Figure 6. Free MPA-AUC_{0-12} values in 18 pediatric (circles) and 10 adult (squares) renal transplant recipients at 1 wk (A) and 3 wk (B) after renal transplantation as a function of total MPA-AUC_{0-12} values. At 3 wk ($r = 0.46$, $P < 0.05$), but not at 1 wk ($r = 0.23$, $P = 0.23$), there was a moderate linear correlation.

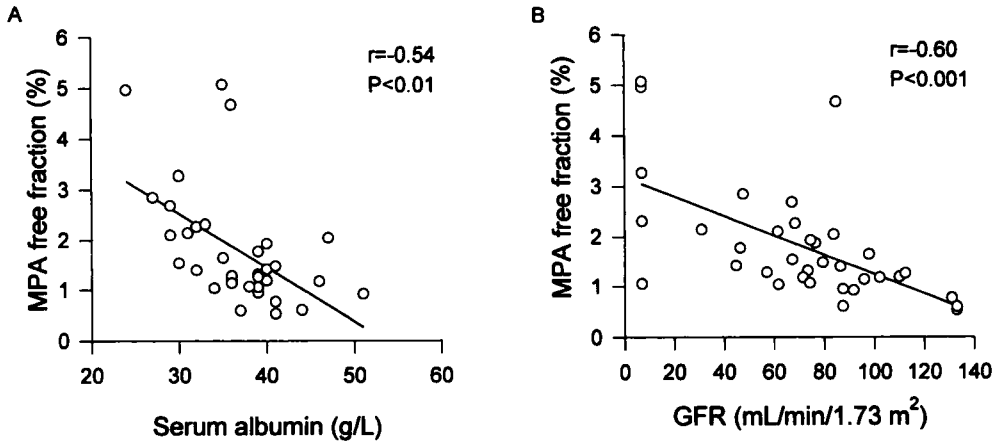

Figure 7. MPA free fraction in pediatric renal transplant recipients ($n = 18$) as a function of serum albumin levels (A) and GFR (B). Data from the investigation at 1 wk and at 3 wk after renal transplantation were combined, resulting in 36 observations.

fraction also had higher free MPA-AUC_{0-12} values, as indicated by a significant linear correlation between the two parameters both at 1 wk ($r = 0.72$, $P < 0.001$) and at 3 wk ($r = 0.80$, $P < 0.001$) after transplantation.

Discussion

According to current data, the pharmacokinetic variable most clearly predictive of immunosuppressive efficacy of MMF is MPA-AUC (9). Therefore, it was important to demonstrate that a dose of 600 mg MMF/m^2 BSA in children between the ages of 6 and 15 yr yielded comparable AUC for MPA and free MPA at 3 wk after renal transplantation as adults who received 1 g of MMF, the recommended twice daily oral dose in adults (18). Hence, the BSA-adjusted dosing of MMF appears to be appropriate in pediatric renal transplant recipients. The dose–AUC relationship in children under the age of 6 remains to be established. Because for MMF there is a relationship between the immunosuppressive efficacy in renal transplant recipients and the MPA-AUC (9), we predict that MMF in a dose of 600 mg/m^2 twice a day displays a comparable effectiveness in pediatric transplant recipients, as observed previously in adult patients. This hypothesis is currently being investigated in a large multicenter study.

There was a considerable inter- and intraindividual variability of pharmacokinetic parameters in both patient groups. Some of this variability appears to be related to the function of the kidney transplant, because in patients with primary transplant dysfunction a lower maximal MPA concentration and a longer time to maximum concentration were observed. This may be due to slower absorption of MMF, most likely as a result of uremic dysmotility of the gastrointestinal tract (19). However, a slower conversion of MMF to MPA in uremic patients cannot be formally excluded. Also, changes in the enterohepatic circulation of MPA might contribute to the variability of MPA-AUC values in the early posttransplant period (9).

Plasma albumin binding of MPA is high, having a mean value in normal plasma of 97% (9). In the present investigation, the median protein binding of MPA 3 wk after transplantation was 98.5% in children and 98.1% in adults. Because *in*

vitro the pharmacologic activity of MPA is a function of unbound drug concentration (11) and uremia is a known cause of altered binding of anionic or acidic drugs to serum albumin (20), we sought to identify factors that might be important in modulating the free fraction of MPA in the first few weeks after renal transplantation. To our knowledge, this is the first report of the pharmacokinetics of free MPA in renal transplant recipients. Despite a 20-fold increase of total MPA, the MPA free fraction did not change significantly throughout the 12-h dosing interval, consistent with previous *in vitro* data (11). However, we observed a significant inverse correlation between the MPA free fraction and serum albumin levels in children. In our study, the MPA free fraction was 1.5% at a physiologic serum albumin of 40 g/L and increased more than twofold at a low serum albumin concentration of 24 g/L (Figure 7). These data compare well with *in vitro* findings in which a 2.2-fold increase in MPA free fraction was observed when the human serum albumin concentration was decreased from its physiologic concentration of 41.4 to 20.7 g/L (11). In addition, GFR was a significant determinant of MPA free fraction. Approximately 50% of the variability of MPA free fraction in children in our study could be explained by a linear combination of serum albumin levels and GFR. The effect of GFR on protein binding of MPA is most likely due to several factors. First, there was a positive correlation between the MPA free fraction and the renal metabolite MPAG, presumably by a competitive displacement mechanism of albumin-bound MPA by high concentrations of MPAG (11). MPAG is bound to 82% to plasma albumin in stable patients after renal transplantation; in patients with renal impairment, MPAG accumulates in plasma and protein binding decreases to approximately 62% (9). Second, patients with reduced transplant function had lower serum albumin levels. Third, the lower plasma pH in patients with kidney transplant dysfunction might alter MPA protein binding, because according to *in vitro* data, a decrease of the plasma pH from 7.4 to 7.0 in a normal human plasma pool increased the MPA free fraction from 1.7 to 2.4% (11). Other possibilities include a change in albumin structure or displacement by endogenous inhibitors that accumulate in renal failure (20).

Theoretically, mainly the free drug is available for metabolism and excretion, and clearance may increase in proportion to the increase in free concentration (20). The net result could be no change in the absolute free concentration of the compound. However, this was not the case, because in our investigation a higher MPA free fraction was associated with an increase in the free MPA concentration–time curve in renal transplant recipients. Studies are in progress to evaluate whether the concentration–time curve of free MPA is a better predictor of the immunosuppressive efficacy and toxicity of MMF than that of total MPA.

In conclusion, pediatric renal transplant recipients in the first 3 wk after surgery display concentration–time profiles of total and free MPA after oral administration of 600 mg MMF/m^2 BSA twice a day that, in general, are comparable to those in adults receiving the currently recommended oral dose of 1000 mg of MMF twice a day. Renal functional impairment had no consistent effect on the MPA-AUC$_{0-12}$ values, but the MPA free fraction was influenced by renal transplant function, MPAG, and serum albumin levels. Because the pharmacologic activity of MPA is a function of unbound drug concentration, we are currently investigating the pharmacodynamic significance of free MPA in renal transplant recipients and the possible relation to clinical outcome events.

Appendix

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