

Concentrations of Mycophenolic Acid and Glucuronide Metabolites Under Concomitant Therapy With Cyclosporine or Tacrolimus

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Abstract: Mycophenolate mofetil [MMF, the prodrug of mycophenolic acid (MPA)] is usually administered at double doses with cyclosporine than with tacrolimus because it is believed that MPA exposure is lower during cyclosporine therapy. This study aimed to compare 12 hour, steady-state concentration–time profiles of MPA and its phenol- and acyl-glucuronide metabolites (MPAG and AcMPAG, respectively) in stable kidney transplant recipients maintained either on cyclosporine (n = 12) or tacrolimus (n = 12). During the absorption phase in the cyclosporine group, dose-normalized concentrations of total and free MPA were significantly higher but the overall area under the concentration–time curve (AUC_{0–12}) was not significantly different. Additionally, exposure to AcMPAG was higher in the cyclosporine group ($P < 0.05$). Ten of 12 patients in the cyclosporine group were on ketoconazole therapy; however, the exposure to MPA or MPAG was not different when MMF was given orally to Sprague-Dawley rats with or without ketoconazole. In conclusion, cyclosporine modulates the disposition of MPA and metabolites differently from tacrolimus; however, patients on cyclosporine may not require double doses of MMF to achieve the same exposure.

Key Words: acyl-MPAG, cyclosporine, ketoconazole, MPAG, mycophenolic acid, pharmacokinetics, tacrolimus

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INTRODUCTION

Mycophenolic acid (MPA) is an inhibitor of inosine monophosphate dehydrogenase commonly used as an immunosuppressant for prevention of allograft rejection.¹ It is administered as an ester prodrug, mycophenolate mofetil

(MMF; CellCept; Roche Laboratories, Inc., Nutley, NJ), or an enteric-coated formulation of mycophenolate sodium (Myfortic; Novartis Pharmaceuticals Corporation, East Hanover, NJ). The absolute oral bioavailability of MPA administered as MMF is greater than 90%² with peak plasma concentrations of MPA occurring within the first hour after oral ingestion. A secondary peak of MPA, indicative of enterohepatic recirculation, is usually observed between 7 and 10 hours postdose in some patients and is the result of the biliary secretion of mycophenolic acid phenyl-glucuronide metabolite (MPAG).² The extent of exposure to MPA as defined by the area under the concentration–time curve (AUC) and is therefore dependent on the extent of intestinal absorption of MMF and MPA reentering the blood after enterohepatic recirculation.²

Investigations into the metabolism of MPA suggest that MPA is largely metabolized by the UDP-glucuronosyltransferase (UGT) system in the kidney followed by the liver and then the intestine.^{3,4} Moreover, UGT1A9 and UGT1A10 systems, expressed primarily in the kidney and to some extent in the liver and intestine, are responsible for the formation of MPAG, whereas UGT2B7 expressed mainly in the liver is responsible for the formation of the pharmacologically active mycophenolic acid acyl-glucuronide metabolite (AcMPAG).^{3,4} Both metabolites are glucuronide conjugates and differ only in the position of the glucuronide moiety in that MPAG is a chemically stable phenolic glucuronide, whereas in AcMPAG, the glucuronide is linked to the molecule through carboxylic acid and therefore is chemically reactive and prone to pH-dependent acyl-migration.⁵

Mycophenolic acid is currently administered in combination therapy with calcineurin inhibitors (CNIs) cyclosporine or tacrolimus and corticosteroids.¹ Although the dose of cyclosporine or tacrolimus is optimized according to blood levels, plasma concentrations of MPA are not routinely monitored and the dose adjustments remain empiric.⁶ In the absence of therapeutic drug monitoring, the possibility of drug–drug interaction becomes of great concern in a sensitive population such as transplant recipients.⁷ It is therefore essential to fully characterize the probability and extent of drug–drug interactions with commonly administered medications in this patient group.

Numerous studies have focused on evaluating the effect of CNIs on the pharmacokinetics of MPA and MPAG. Zucker and colleagues⁸ observed that MPA trough concentrations and AUC were higher in tacrolimus-treated patients, whereas the concentration of MPAG was lower in these patients. Another

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publication by the same researchers showed that in the presence of tacrolimus, glucuronidation of MPA to MPAG was reduced in isolated liver and kidney microsomal systems.⁹ Similarly, a study by Naito and colleagues¹⁰ in Japanese kidney transplant recipients have reported higher MPAG levels in cyclosporine-treated patients for samples obtained before MMF dose ($t = 0$) and 2 hours after dose ($t = 2$), although there were no differences in the MPA concentrations. Based on these studies, many transplant centers now administer MMF at double daily doses in patients on cyclosporine coadministration than in those on tacrolimus therapy.

To elucidate the mechanism of drug interaction between cyclosporine and tacrolimus with MPA, disposition of MPA was studied in Sprague-Dawley and Eisai hyperbilirubinemic (EHB) rats.¹¹ The EHB rats have a mutation in the gene responsible for the expression of multidrug resistance-associated protein 2 (Mrp2) or canalicular multispecific organic anion transporters (cMOAT). The study showed that MPAG was a substrate for Mrp2 and its biliary excretion was inhibited by cyclosporine but not tacrolimus.¹¹ Additionally, in another study in Mrp2-deficient rats, the concentration–time profiles of MPA and MPAG were identical under cyclosporine or tacrolimus coadministration, which further supports the notion of differential modulation of Mrp2 transport proteins by cyclosporine or tacrolimus.¹² Thus, there are 2 possible scenarios that can be presented from the observations of these investigations, one being the higher levels of MPA and lower levels of MPAG in patients with tacrolimus coadministration may be the result of the inhibition of MPA glucuronidation by tacrolimus or the second being the inhibition of the Mrp2-mediated hepatic disposition of MPAG by cyclosporine.

Although the pharmacokinetic characteristics of MMF during concomitant administration of either cyclosporine or tacrolimus have been studied previously,^{8,12–16} the effects of cyclosporine or tacrolimus coadministration on the concentration of MPA metabolites, more specifically the pharmacologically active AcMPAG, is not known. We recently completed a pharmacokinetic study in stable kidney transplant recipients¹⁷ to characterize the concentration–time profiles of total and free MPA and its metabolites MPAG and AcMPAG in patients with diabetes. This study was specifically designed to include an equal number of patients on cyclosporine or tacrolimus.¹⁷ The current article presents the results of an investigation on the total MPA, MPAG, and AcMPAG and unbound MPA concentrations in stable kidney transplant recipients under chronic therapy with either cyclosporine or tacrolimus and a pharmacokinetic study in rats to characterize the possibility of an interaction between MMF and ketoconazole.

METHODS

Human Subjects

An open-label evaluation of MPA pharmacokinetics in stable kidney transplant recipients was performed¹⁷ after receiving approval from the Lifespan Institutional Review Board and the Institutional Review Board at the University of Rhode Island. All clinical investigations were carried out at the Clinical Research Facility at the Hallet Center for Diabetes and

Endocrinology Diseases (Rhode Island Hospital, Providence, RI) or the offices of Transplant Services at Rhode Island Hospital (Providence, RI). Patients were required to fast overnight and arrive at the center by 7:00 AM at which time they underwent physical examination and baseline urine and blood samples were collected. Patients were served 3 standard diabetic hospital meals consisting of 2000 Kcal per day at 10:00 AM, 1:00 PM, and 7:00 PM. The patient recruitment was designed so that at the completion of the study, there were equal numbers of patients in the cyclosporine and tacrolimus groups ($n = 12$ in each group) with roughly equal numbers of patients being diabetic or nondiabetic (Table 1). Patients under the age of 18 years, patients who received an allograft less than 3 months before the study, and pregnant or nursing females were excluded as were patients with evidence of severe liver disease as defined by liver enzyme levels >3 times the upper limit of normal.

Immunosuppressive Therapy

According to the protocol for immunosuppressive therapy, patients were on concomitant therapy with either cyclosporine or tacrolimus in addition to oral prednisone. Patients on cyclosporine were initially administered 1000 mg MMF (CellCept) twice daily and patients on tacrolimus were administered 500 mg MMF twice daily. However, MMF dosage is often adjusted if the patient exhibits MPA-related side effects, including diarrhea or leukopenia. Cyclosporine or tacrolimus dosage is also adjusted according to the routinely measured whole blood trough concentrations of these drugs.

TABLE 1. Demographic Characteristics of the Patients

	Cyclosporine (n = 12)	Tacrolimus (n = 12)
Age (years)	53.3 ± 13.7	54.6 ± 7.0
Age range	25–72	40–62
Body weight (kg)	89.1 ± 19.5	82.6 ± 18.8
Gender (male/female)	10/2	12/0
Ethnicity (white/other)	10/2	12/0
Months posttransplant (range)	9.4–72.0	5.0–76.5
Etiology of renal dysfunction		
Glomerular disorders	5	3
Polycystic kidney disease	1	2
Hypertensive renal disease	1	1
Diabetic renal disease	5	6
Number of diabetic patients/nondiabetic patients	6/6	7/5
Creatinine (g/dL)	1.5 ± 0.2	1.3 ± 0.5
Glomerular filtration rate (mL/min/1.73 m ²)*	40.2 ± 16.7	51.2 ± 22.7
Glucose (mg/dL)	125.3 ± 91.2	120.6 ± 47.8
Albumin (g/dL)	4.3 ± 0.3	4.2 ± 0.3
Alanine transaminase (U/L)	8.7 ± 4.7	10.3 ± 4.8
Aspartate transaminase (U/L)	23.0 ± 7.0	19.2 ± 4.4
Bilirubin (mg/dL)	0.3 ± 0.1	0.3 ± 0.2

*Glomerular filtration rate (GFR) was measured using iothexol clearance method. Unless otherwise stated, all values are expressed as mean ± standard deviation.

In this transplant center, patients on cyclosporine were routinely administered ketoconazole to reduce the cyclosporine dosage requirement and the cost of therapy.¹⁸ The dose of ketoconazole is usually 200 mg per day unless the dose of cyclosporine needs to be lowered to 25 mg twice daily to achieve a desired therapeutic level, in which case ketoconazole dose is reduced to 100 mg per day.

Sample Collection Schedule

At the beginning of a study day, polyethylene catheters were inserted into the cubital vein of each arm and a predose blood sample was obtained. The patients were then administered their usual dose of MMF with a glass of water (250 mL) along with their respective doses of cyclosporine or tacrolimus and any other medications usually taken in the morning. Blood samples were subsequently collected at 0.25, 0.5, 1.0, 1.5, 2, 3, 4, 5, 7, 9, 10, and 12 hours after the dose and the exact time of blood collection was recorded. The blood samples were immediately centrifuged at $1500 \times g$ and plasma was stored at -80°C until analysis.

Pharmacokinetic Study in Rat

Materials

Oral suspension of 200 mg/mL (CellCept; Roche Laboratories Inc., Nutley, NJ) was donated by Roche Laboratories. Sterile water for injection [United States Pharmacopeia (USP)], isotonic saline solution, 1- and 3-mL syringes, 20- and 22-gauge needles, glycerol, and heparin sodium injection (1000 USP units/mL) were purchased from the State Pharmacy (Warwick, RI). Ketoconazole powder (Sigma, St. Louis, MO) was dissolved in 50:50 v/v ethanol:polyethylene glycol to give a final concentration of 10 mg/mL. Oral gavage smooth jaw Webster needles were purchased from Kent Scientific Corporation (Torrington, CT). StatSpin microtubes with gel were purchased from StatSpin Inc (Norwood, MA). Drug-free rat serum was purchased from Harlan (Indianapolis, IN).

Animals

The Institutional Animal Care and Use Committee at the University of Rhode Island (IACUC No. AN05-06-031) approved the study. Adult male Sprague-Dawley rats (average weight 250 g) with jugular vein cannulation maintained as a subcutaneous pouch were purchased from Harlan (Indianapolis, IN). The rats ($n = 16$) were housed in individual cages and had free access to food and water before the study; however, the rat feed was removed the night before the pharmacokinetic study. The rats were divided and grouped into equal numbers in each the control (8 rats on MMF only) and test groups (8 rats on MMF + ketoconazole). To achieve steady-state concentrations of drugs in both groups, the rats were dosed once a day consecutively for 3 days before the study day. The control group was administered a suspension containing 5 mg MMF (0.25 mL), whereas the test group was administered 5 mg MMF and 2.5 mg ketoconazole by oral gavage. On any given study day, each rat was weighed before drug administration and a 0.15 mL baseline blood sample ($t = 0$) was obtained. The previously mentioned drug doses were then administered by oral gavage and 0.15 mL blood

samples were obtained subsequently at 0.5, 1, 3, 5, 7, 9, and 12 hours after the dose. After each sampling, the patency of the cannulas was maintained by flushing with 0.15 mL sterile isotonic saline followed by slowly injecting 0.1 mL sterile heparinized glycerol (500 IU heparin/mL) to fill the cannula lining. The blood samples were transferred to StatSpin microtubes, allowed to stay in contact with the gel in the tubes for 30 minutes before centrifugation at $10,000 \times g$ for 2 minutes, and the serum samples were stored at -80°C until analysis.

Analytic Methods

Samples From Human Pharmacokinetic Study

The concentration of total MPA, MPAG, and AcMPAG was determined using a high-performance liquid chromatography with ultraviolet detection (HPLC-UV) method with a lower limit of quantification (LLOQ) of 0.2, 2.0, and 0.5 mg/L, respectively. The percent coefficient of variation for the low, medium, and high concentration of the quality control samples was 7.6%, 4.1%, and 6.1% for total MPA, 4.6, 4.0 and 8.0 for AcMPAG, and 5.3, 4.7 and 5.0 for MPAG.¹⁹ The unbound concentration of MPA was analyzed by ultrafiltration of plasma followed by a validated liquid chromatography tandem mass spectrometry (LC-MS/MS) method with a LLOQ of $1 \mu\text{g/L}$ with the percent of coefficient of variation of 4.0%, 10.6%, and 2.3%, respectively.²⁰

Samples From Rat Pharmacokinetic Study

Rat serum samples were analyzed by a modified version of the HPLC-UV method used for human studies.¹⁹ Combined internal standard solution containing 25 mg/L each of phenolphthalein glucuronic acid (internal standard for MPAG) and carboxy butoxy ether of MPA were prepared in methanol (internal standard for MPA and AcMPAG). Five calibrators and 2 quality controls containing MPA, MPAG, and AcMPAG were prepared in rat serum. The 5 calibrators contained 0.2, 1.0, 5.0, 10, and 25 mg/L MPA; 0.5, 2.5, 5.0, 10, and 15 mg/L AcMPAG; and 2, 10, 25, 150, and 250 mg/L MPAG, whereas the quality controls contained 0.5 and 30 mg/L MPA, 0.75 and 20 mg/L AcMPAG, and 5 and 300 mg/L MPAG. To 50 μL of the serum sample, 100 μL of the combined internal standard solution prepared in methanol was added to precipitate the proteins. The samples were then vortex mixed for 30 seconds and centrifuged at $10,000 \times g$ for 10 minutes. To 100 μL of the supernatant, 50 μL of 0.1% v/v phosphoric acid was added. The samples (50 μL) were then injected onto the analytic column and absorbance was measured at UV wavelength of 254 nm.

Determination of Des-methyl MPA Concentrations

We have attempted to measure the des-methyl MPA (DM-MPA) concentrations using a recently developed LC-MS/MS analytic method for quantification of DM-MPA. The instrumentation included an HPLC (Perkin Elmer, Norwalk, CT) and an API2000 triple quadrupole mass spectrometer (Sciex, Toronto, CA). Des-methyl MPA standard was obtained from Roche Biosciences (Palo Alto, CA). Sample preparation included extraction of DM-MPA from calibrator or patient plasma sample (100 μL) by adding 200 μL of methanol, vortexing the sample for 5 minutes, and centrifuging at $10,000 \times g$

for 10 minutes. For chromatographic purposes, 20 μL of deionized water was added and the sample was recentrifuged at $10,000 \times g$ for 5 minutes. The supernatant (250 μL) was then transferred into HPLC vials and 15 μL was injected onto a Zorbax C₈ 150 \times 4.6 mm (5 μm particle size) analytic column (Agilent Technologies, Palo Alto, CA). The chromatographic conditions were those used in a previously published assay²⁰ with indomethacin as the internal standard. The mass breakdowns selected for DM-MPA were m/z : 305.0 \rightarrow 261.2 in first and third quadrupole, respectively, and acquisition was performed in negative ionization mode. The assay was linear in the concentration range of 2.5 to 1000 $\mu\text{g/L}$ and the LLOQ was 2.5 $\mu\text{g/L}$ for DM-MPA.

Data Analysis

Data from both human and rat studies were analyzed using the noncompartmental method on WinNonlin software version 4.1 (Pharsight, Mountain View, CA). Parameters included area under the concentration–time curve from 0 to 12 hours (AUC_{0-12}), minimum plasma concentration (C_{min}), maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), and apparent clearance (for MPA only). When dose-normalized values are reported, plasma concentration or the pharmacokinetic parameters were normalized to a dose of 1000 mg MMF twice daily.

All statistical tests were performed using SPSS software version 11.5 (SPSS, Chicago, IL) and a P value < 0.05 was considered significant. To establish normal distribution, Shapiro-Wilk statistics were calculated for all parameters. Nonnormally distributed data were reported as median and interquartile range and were analyzed using appropriate nonparametric methods. Independent-sample t test or Mann-Whitney U tests were used to compare the differences in parameters between cyclosporine and tacrolimus groups for normal and nonnormally distributed data, respectively. The differences in proportions were calculated using Pearson χ^2 test.

RESULTS

Demography and Pharmacotherapy

A total of 26 patients were enrolled; however, 2 patients discontinued because of difficult venous access. The demographic characteristics of patient population are shown in Table 1. Patient age, weight, gender, and ethnic origin was similar between the 2 groups. In addition, etiology of kidney dysfunction, length of time after organ transplantation, and the number of patients with diabetes were comparable (Table 1). Kidney function was slightly better in the tacrolimus group as characterized by lower serum creatinine concentration and higher iothexol clearance, but this difference was not statistically significant. Other biochemical indices, including serum glucose, albumin, and liver function tests (alanine transaminase, aspartate transaminase, and bilirubin) were similar between the 2 groups (Table 1).

The dosing regimen for immunosuppressive agents and other medications is summarized in Table 2, including the number of patients receiving MMF at each dose level (250, 500, and 1000 mg twice daily). The daily dose of MMF was

TABLE 2. Dosing Regimen of Immunosuppressive Agents and Other Medications

	Cyclosporine (n = 12)	Tacrolimus (n = 12)	P Value
MMF dose (mg/day)	1583.2 \pm 514.9	1041.6 \pm 334.2	0.007
No. of patients/MMF dose level (mg twice daily)			
250	0	1	0.028
500	5	10	
1000	7	1	
Cyclosporine dose (mg/day)	115 \pm 105	—	—
Cyclosporine level ($\mu\text{g/L}$)*	95 \pm 50	—	—
Tacrolimus dose (mg/day)	—	7 \pm 5	—
Tacrolimus level ($\mu\text{g/L}$)*	—	10 \pm 4	—
Prednisone dose (mg/day)	6.0 \pm 2.2	6.9 \pm 3.7	0.45
No. of patients on lipid-lowering agents (atorvastatin/pravastatin)	9	7	NS
No. of patients on insulin	4	3	NS
No. of patients on warfarin	0	2	NS
No. of patients on ketoconazole (100–200 mg/day)	10	0	< 0.0001

NS, not significant.

*Both cyclosporine and tacrolimus levels represent morning trough blood concentration measured using LC-MS/MS.

significantly higher in the cyclosporine group than the tacrolimus group, which is a reflection of the routine practice of starting 2000 mg MMF per day to patients on cyclosporine as compared with 1000 mg per day to patients on tacrolimus. The average whole blood trough concentration of cyclosporine was $95 \pm 50 \mu\text{g/L}$ on a cyclosporine dose of $115 \pm 105 \text{ mg per day}$ versus a tacrolimus level of $10 \pm 4 \mu\text{g/L}$ on a tacrolimus dose of $7 \pm 5 \text{ mg per day}$. The daily dose of prednisone and the number of patients on lipid-lowering agents (atorvastatin or pravastatin), insulin, or warfarin were similar between the 2 groups. The only major difference in drug therapy was a significantly larger number of patients in the cyclosporine group (10 of 12) were also receiving ketoconazole versus no such patient in the tacrolimus group (Table 2).

Pharmacokinetics of MPA, MPAG, and AcMPAG

Primary pharmacokinetic parameters, including time for maximum plasma concentration (T_{max}), minimum concentration (C_{min}), maximum concentration (C_{max}), and area under the concentration–time curve (AUC_{0-12}), for total and unbound MPA, MPAG, and AcMPAG is shown in Table 3. Considering the daily dose of MMF was higher in patients on cyclosporine, all nondose-normalized parameters were also significantly higher (Table 3) with the exception of MPA trough concentration (C_{min}) that was only marginally higher ($P = 0.110$).

Comparison of dose-normalized values revealed that unexpectedly all parameters were still higher for cyclosporine-treated patients, but considering wide interpatient variation,

TABLE 3. Comparison of Minimum Concentration (C_{\min}), Maximum Concentration (C_{\max}), and Area Under The concentration–time Curve (AUC_{0-12}) for Total and Unbound MPA as Well as MPAG and Acyl-MPAG (Each Group Consists of 12 Patients)

	Parameters			Dose-normalized Parameters*		
	Cyclosporine	Tacrolimus	<i>P</i> Value	Cyclosporine	Tacrolimus	<i>P</i> Value
Total MPA						
T_{\max} (hr)	1.1 (1.0–1.6)	1.0 (0.5–1.6)	0.410			
C_{\min} (mg/L)	0.7 (0.5–2.3)	0.5 (0.3–1.1)	0.110	1.3 (0.8–2.3)	1.0 (0.6–2.0)	0.551
C_{\max} (mg/L)	13.1 (6.5–19.0)	6.0 (4.1–9.0)	0.028	15.5 (11.2–19.1)	12.9 (9.5–17.3)	0.291
AUC_{0-12} (mg*hr/L)	44 (24–61)	23 (18–32)	0.028	60 (39–87)	42 (35–63)	0.268
Apparent clearance (L/hr)	12.1 (8.4–19.0)	17.4 (11.7–20.9)	0.248			
Unbound MPA						
T_{\max} (hr)	1.1 (0.8–1.9)	1.1 (0.6–2.0)	0.770			
C_{\min} (μ g/L)	13.6 (9.6–14.4)	5.6 (5.0–8.7)	0.000	16.1 (13.8–21.1)	12.2 (9.9–18.0)	0.089
C_{\max} (μ g/L)	94.4 (60.1–232.6)	57.6 (42.7–82.0)	0.020	128.5 (94.7–242.0)	115.2 (86.5–163.9)	0.410
AUC_{0-12} (μ g*hr/L)	374 (315–431)	204 (151–251)	0.000	571 (407–766)	407 (335–502)	0.114
Fraction unbound (fu%)	0.94 (0.59–1.64)	0.90 (0.60–1.37)	0.330			
MPAG						
T_{\max} (hr)	2.2 (1.3–4.0)	2.0 (1.5–2.1)	0.510			
C_{\min} (mg/L)	48.1 (33.2–76.7)	28.7 (17.5–43.0)	0.007	78.9 (41.1–98.9)	53.8 (37.4–86.1)	0.319
C_{\max} (mg/L)	100.9 (71.2–153.6)	57.8 (43.4–72.7)	0.001	139.2 (108.5–185.3)	103.8 (84.3–145.3)	0.101
AUC_{0-12} (mg*hr/L)	806 (653–1405)	487 (369–718)	0.003	1259 (921–1514)	933 (718–1436)	0.189
Acyl-MPAG						
T_{\max} (hr)	2.6 (1.9–4.4)	2.1 (1.6–3.0)	0.440			
C_{\min} (mg/L)	1.6 (1.2–2.2)	0.8 (0.5–1.2)	0.001	2.1 (1.5–2.8)	1.5 (1.1–2.2)	0.178
C_{\max} (mg/L)	3.8 (2.9–4.6)	1.7 (1.1–2.4)	0.000	5.0 (3.7–7.2)	3.3 (2.2–4.4)	0.020
AUC_{0-12} (mg*hr/L)	31 (23–46)	14 (10–19)	0.000	47 (33–57)	27 (21–38)	0.050

*Dose-normalized parameters are calculated based on a 1000 mg dose of MPA. Values are expressed as median (interquartile range).

the differences were not statistically significant for the majority of parameters. Indeed, median values of dose-normalized AUC_{0-12} for total MPA was 42%, unbound MPA was 40%, MPAG was 35%, and AcMPAG was 74% higher in the cyclosporine group. Furthermore, dose normalized C_{\max} and AUC_{0-12} values for AcMPAG were significantly higher in the cyclosporine-treated patients. Moreover, 9 of 12 patients in the cyclosporine group showed absence of enterohepatic recirculation compared with only 2 of the 12 patients in the tacrolimus group.

Figures 1A–D represents dose-normalized concentration–time profiles of MPA and related compounds. Total MPA concentrations were significantly higher around the MPA peak (at 1.5 hours postdose) (Fig. 1A) and the concentration of unbound MPA was significantly higher at 1.5, 5, and 10 hours after the dose (Fig. 1B) in the cyclosporine group. The concentrations of MPAG, although higher, were not significantly different at any time point after the dose, whereas the concentrations of AcMPAG were significantly higher at 1, 1.5, 3, 4, 5, 7, and 9 hours in the cyclosporine group.

Comparison of MPA Pharmacokinetics With Respect to Diabetes

Data were divided into 2 groups with respect to the diabetic status of the patients. The concentration–time profiles

and other dose normalized pharmacokinetic parameters were compared between the cyclosporine (6 diabetics, 6 nondiabetics) and tacrolimus (7 diabetics, 5 nondiabetics) groups. In patients with diabetes, MPA T_{\max} was marginally delayed in patients on cyclosporine (T_{\max} 1.7 ± 0.8 vs 1.2 ± 0.5 hours, cyclosporine vs tacrolimus, respectively, $P = 0.19$); however, MPA T_{\max} was comparable (less than 1 hour for both groups) in nondiabetic patients. C_{\max} values for MPA, fMPA, and MPAG was similar between cyclosporine and tacrolimus groups with respect to the diabetic status. The AcMPAG C_{\max} , however, was not different between the 2 groups only in diabetic patients ($P = 0.34$) but significantly higher in nondiabetic patients on cyclosporine (5.6 ± 1.3 vs 3.1 ± 1.3 mg/L in cyclosporine vs tacrolimus respectively, $P = 0.011$). Similarly, the AUC_{0-12} for all parameters were similar; however, the AUC_{0-12} of AcMPAG was higher in nondiabetic patients on cyclosporine (48 ± 1.2 vs 26 ± 1.6 mg*hr/L, cyclosporine vs tacrolimus, respectively, $P = 0.021$).

Metabolite Ratio in Cyclosporine and Tacrolimus-Treated Patients

Figures 2A–B illustrate the ratio of MPA active (AcMPAG) to inactive (MPAG) metabolites (multiplied by 100) over the 12 hour dosing interval in patients on cyclosporine (Fig. 2A) or tacrolimus (Fig. 2B). The figure

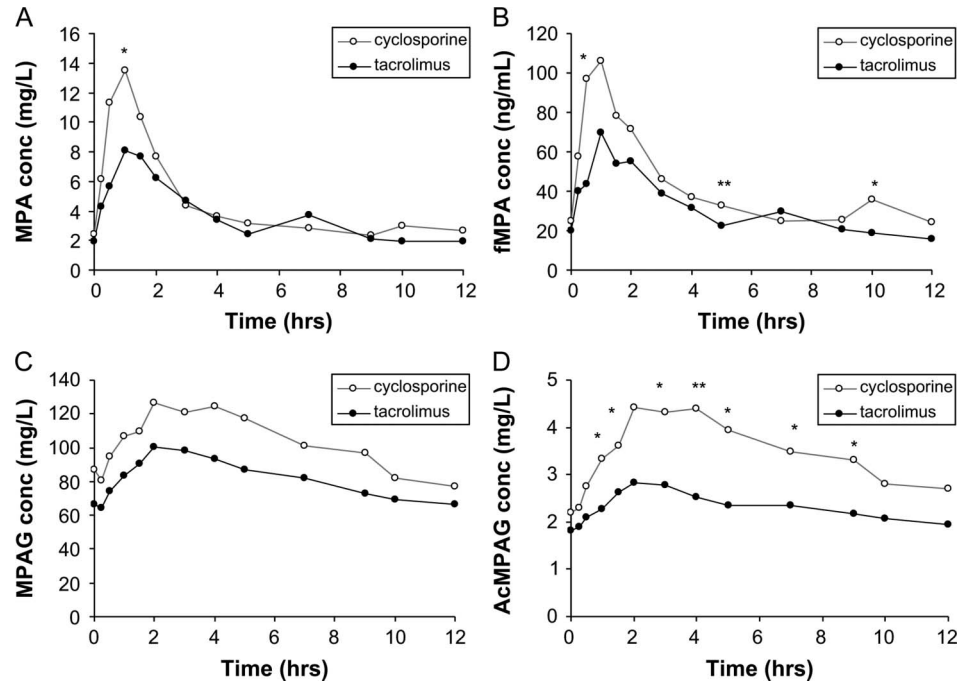


FIGURE 1. (A–D) Dose-normalized concentration–time profiles of (A) total MPA, (B) unbound MPA (fMPA), (C) MPAG, and (D) acyl-MPAG for patients in the cyclosporine (n = 12) and tacrolimus groups (n = 12). Concentrations are normalized to a dose of 1000 mg mycophenolate mofetil. Asterisks represent the level of significance between the 2 groups (*P < 0.05 and **P < 0.01).

insets depict total MPA concentrations versus the AcMPAG to MPAG ratios for cyclosporine (Fig. 2A inset) and tacrolimus (Fig. 2B inset) groups as a function of MPA concentration. The figures were plotted to illustrate that MPA metabolism to MPAG is likely to reach saturation in higher MPA concentrations in the cyclosporine group, thus favoring the formation of AcMPAG during peak MPA concentrations. The pattern for the ratio of AcMPAG to MPAG is also different between the cyclosporine or tacrolimus groups. For example, the metabolite ratio gradually increases in cyclosporine-treated patients as the plasma concentrations of MPA increases. This favored metabolism is likely to be carried through the remaining sampling period, even when MPA concentrations start to drop toward the end of the sampling time points. This is clearly illustrated in the inset for Figure 2A, in which the ratio keeps increasing despite a drop in plasma MPA concentrations. As shown in the inset of Figure 2B, this ratio remains constant throughout the sampling period in tacrolimus-treated patients.

Determination of DM-MPA Concentrations

We have attempted to measure the concentration of phase I metabolite of MPA (DM-MPA) in plasma samples from humans before dose and around peak plasma concentration of MPA. No trace of DM-MPA in the majority of samples was observed or the concentration was below the level of quantification (2.5 µg/L) for the assay. This prevented acquiring data pertinent to the phase I metabolism of MPA.

Drug Interaction Study Between MMF and Ketoconazole in Rats

The average weight of the rats in the MMF group was 251.5 ± 15.1 g, whereas the weight of the rats in the MMF + ketoconazole group was 239.4 ± 11.1 g (P = 0.16). Cannula patency was lost in 7 rats shortly after the start of pharmacokinetic study; hence, the serum concentrations from rats with incomplete pharmacokinetic profiles were excluded from the final analysis. None of the rats showed formation of AcMPAG, and the data on total MPA and MPAG

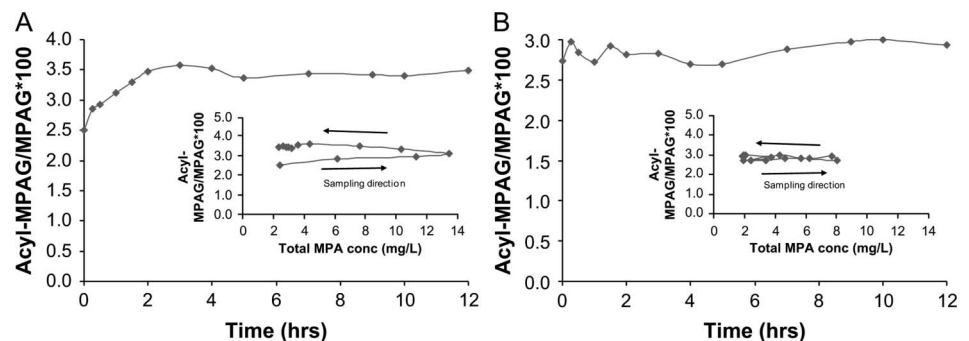


FIGURE 2. (A–B) Plot of time versus metabolite concentration ratios (acyl-MPAG/MPAG) for patients in the (A) cyclosporine group or the (B) tacrolimus group. The insets represent the association between total MPA concentrations versus the metabolite ratio.

concentrations from a total of 11 rats, 4 rats in the MMF group and 7 rats in the MMF + ketoconazole group, has been reported. A comparison of the basic pharmacokinetic parameters (C_{\min} , C_{\max} , AUC_{0-12}) for total MPA and MPAG is shown in Table 4. None of the pharmacokinetic parameters were significantly different between the 2 groups indicating lack of drug–drug interaction between MMF and ketoconazole in this animal model.

DISCUSSION

We present the results of a clinical pharmacokinetic investigation that evaluates the influence of concurrent treatment with the calcineurin inhibitors cyclosporine or tacrolimus on the concentration–time profile of MPA and the metabolites, MPAG and AcMPAG. Although the study was originally designed to compare MPA pharmacokinetics in diabetic and nondiabetic kidney transplant recipients,¹⁷ special care was taken to recruit an equal number of patients on cyclosporine and those on tacrolimus with similar demographic characteristics. In this way, we were able to compare the pharmacokinetic parameters of MPA and related compounds under concomitant therapy with either cyclosporine or tacrolimus.

It is commonly believed that transplant patients on cyclosporine have lower exposure to MPA as compared with patients on tacrolimus; therefore, MMF is administered at twice the daily doses in comparison with patients on tacrolimus. Our study was also aimed to characterize the concentration of 2 major metabolites of MPA, MPAG, and AcMPAG as well as a phase I metabolite DM-MPA. Unexpectedly, we have found that in the patients studied, dose-normalized exposure to both total and unbound MPA was somewhat higher in the cyclosporine-treated patients, especially during the absorption phase of MMF and the exposure to the pharmacologically active metabolite of MPA, AcMPAG was also significantly higher in the cyclosporine group.

One possible explanation for our atypical finding of higher MPA concentrations in the cyclosporine group may be related to the fact that 10 of 12 patients were also on concomitant ketoconazole therapy. Ketoconazole is a potent cytochrome P450 (CYP450) inhibitor that is used for its antifungal properties and cyclosporine-sparing effects.^{18,21}

Although MPA is predominantly metabolized by the UGT system, which is a phase II metabolism, a recent report²² indicates that MPA also undergoes phase I metabolism by the cytochrome P450 system enzyme CYP3A4. Apparently an intermediate metabolite, 6-O-desmethyl-MPA (DM-MPA), is formed that is converted to phenol or acyl-glucuronide metabolites of DM-MPA. Using a human liver microsomal assay, these researchers have shown that ketoconazole inhibits the DM-MPA formation by an average of 50%.²² The mentioned publication is the only available report on the effects of ketoconazole on MPA metabolism and no other study in human, animal, or cell culture is currently available to describe the influence of azole antifungal agents on the MPA pharmacokinetics.²² By inhibiting phase I metabolism of MPA, ketoconazole may be responsible for the elevated plasma concentrations of total and unbound MPA seen in the cyclosporine group during the absorption phase.

We have also attempted to measure the plasma concentrations of a phase I MPA metabolite, DM-MPA. However, we could not quantify DM-MPA at trough or even at 1 hour postdose (around MPA T_{\max}) in any of the 24 patients studied. In fact, only a few of the samples showed traces of DM-MPA but the levels were below 2.5 $\mu\text{g/L}$, the lower limit of quantification for our assay. It appears that in humans, either DM-MPA is produced at very low concentrations or is converted rapidly and almost completely to the conjugated form and is therefore not detectable in plasma. The unknown stability of DM-MPA could be another reason for not being able to detect this metabolite in patients' plasma.²²

We have then attempted to dissect the problem by investigating the possibility of drug–drug interactions between MMF and ketoconazole after oral administration in rats. We did not find a difference between primary pharmacokinetic parameters (C_{\max} , T_{\max} , and AUC_{0-12}) indicating lack of an appreciable degree of interaction between ketoconazole and MMF in this animal model. Miles and colleagues²³ have recently shown the UGT enzymes responsible for metabolism of MPA to MPAG differs between human and rat liver microsomes and in general rat liver have lower metabolizing activity than human liver; however, the mentioned study did not investigate metabolism of MPA to AcMPAG.²³ Interestingly, in rats, we have not observed detectable concentrations of AcMPAG in either of the groups, which may indicate lack of production of AcMPAG or rapid and complete hydrolysis to MPA. The lack of interaction between MMF and ketoconazole observed in rats requires further characterization in the context of a drug interaction study in humans.

The mechanism of differential drug interaction between cyclosporine and tacrolimus may also be related to the effect of these agents on the transport proteins, including Mrp2 or multidrug resistance protein 1 (Pgp). Cyclosporine is a documented inhibitor of the transport protein, Mrp2,¹¹ inhibiting the active flux of glucuronide metabolites mediated by Mrp2 and MPAG is a substrate for this transport system. This explains the reduced or lack of enterohepatic recirculation in the cyclosporine-treated patients.^{11,12} It is not known whether MMF is a substrate for Pgp; however, it can be hypothesized that cyclosporine, ketoconazole, or a combination of the two drugs²⁴ may inhibit Pgp or other transporter-mediated efflux

TABLE 4. Comparison of Pharmacokinetic Parameters for Total MPA and MPAG in Sprague-Dawley Rats Given Oral Doses of MMF or MMF + Ketoconazole

	MMF only (n = 4)	MMF + Ketoconazole (n = 7)	P Value
Total MPA			
C_{\min} (mg/L)	1.66 ± 1.05	2.64 ± 1.72	0.30
C_{\max} (mg/L)	2.87 ± 1.61	2.27 ± 0.76	0.83
AUC_{0-12} (mg*hr/L)	22.97 ± 14.39	18.32 ± 11.82	0.57
MPAG			
C_{\min} (mg/L)	2.65 ± 0.70	3.54 ± 0.96	0.09
C_{\max} (mg/L)	3.92 ± 1.29	4.73 ± 2.36	0.55
AUC_{0-12} (mg*hr/L)	31.57 ± 10.95	34.30 ± 10.96	0.70

in the intestine and probably increase the oral absorption of MMF and MPA concentrations during the absorption phase.

AcMPAG metabolite of MPA is pharmacologically active and is likely to be responsible for some of the MPA-related side effects.^{5,25} We have also found that the values of dose-normalized C_{max} and AUC_{0-12} for AcMPAG were significantly higher in cyclosporine-treated patients with higher concentrations throughout the 12 hour sampling period. This observation is consistent with a similar study that found AcMPAG AUC_{0-12} was 38% higher in the patients on cyclosporine than on tacrolimus coadministration.²⁶ We have also observed that the pattern for the ratio of 2 metabolites differed between cyclosporine- and tacrolimus-treated patients. The plots of AcMPAG to MPAG ratio (AcMPAG/MPAG) versus time or total MPA concentration were used to illustrate the possible metabolic saturation of UGT system and to explain the reason for higher AcMPAG concentrations in cyclosporine-treated patients. It appears that in the cyclosporine group, AcMPAG/MPAG increases constantly over the sampling time period even when plasma MPA concentrations tend to drop toward the end time points of sample collection, whereas it remains stable in tacrolimus-treated patients. A possible explanation for this may be the fact that MMF dose was significantly higher in cyclosporine patients and even when dose-normalized concentrations were considered, the MPA levels were appreciably higher at least in the absorption phase. This may be attributable to saturation of enzymes responsible for the formation of MPAG resulting from higher total MPA concentrations, thereby favoring the formation of AcMPAG in the cyclosporine-treated group. Although AcMPAG is not known to be eliminated by active biliary secretion, the influence of cyclosporine and/or ketoconazole on transporter-mediated (Mrp2) disposition of AcMPAG cannot be ruled out and requires further investigation.

In conclusion, this study evaluated the influence of calcineurin inhibitors cyclosporine and tacrolimus on the pharmacokinetics of total and unbound MPA as well as MPA metabolites in 24 stable kidney transplant recipients. It was observed that dose-normalized MPA pharmacokinetic parameters were higher but not significantly different in cyclosporine-treated patients. This can be related to the fact that 10 of 12 patients on cyclosporine were also receiving ketoconazole, a potent inhibitor of CYP3A4 and Pgp, although the interaction between azole antifungal agents with MPA has not been reported before. The fact that transplant recipients receive a cocktail of immunosuppressants and other medications makes determination of drug interactions ambiguous using clinical pharmacokinetic studies. Such interactions can be also studied by the use of valid in vitro cell line based or in vivo animal models in which unknown interactions by other medications can be eliminated. In the context of MPA therapeutic monitoring, elucidation of the drug interactions may help in explaining the large intra- and interindividual variability observed in the pharmacokinetic parameters of this drug. Based on the result of this study, further human pharmacokinetic studies are needed to identify the possible interaction between MMF and ketoconazole or other azole antifungal agents.

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