

Pharmacokinetics of Mycophenolic Acid and Metabolites in Diabetic Kidney Transplant Recipients

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Abstract: Mycophenolate mofetil (MMF), the prodrug of mycophenolic acid (MPA), is an immunosuppressive agent commonly used after organ transplantation. Because diabetes mellitus may affect disposition of pharmacologic agents, we investigated the influence of diabetes on the pharmacokinetics of MPA, unbound MPA (fMPA) and its phenyl and acyl glucuronide metabolites (MPAG and AcMPAG respectively). The study included 13 diabetic and 11 nondiabetic, stable, kidney-transplant recipients who were receiving a triple maintenance immunosuppressive regimen. Serial plasma samples were obtained predose and at regular intervals for 12 hours. Gastric emptying was assessed using an acetaminophen absorption test and glomerular filtration rate was estimated using iohexol clearance. Treatment groups were well matched. The time to maximum concentration (Tmax) of MPA was 86.4 ± 41.4 minutes versus 52.8 ± 31.8 minutes in D and ND patients respectively ($P = 0.04$) indicating a delay in MMF absorption. Neither the maximum MPA concentration nor the 0- to 12-hour area under the concentration-time curve were different. All parameters derived for fMPA and the MPA metabolites were comparable between the 2 groups, except for the metabolite ratio of MPAG and AcMPAG, which was higher for diabetic patients ($P = 0.03$). Delayed gastric emptying seemed to have reduced the initial rate but not the extent of MPA absorption in diabetic patients. The profiles of fMPA were similar in both patient groups. With the exception of metabolite concentration ratio, none of the other parameters associated with MPA metabolism were different between the 2 groups.

Key Words: mycophenolic acid, diabetes, pharmacokinetics, acyl-MPAG, acetaminophen

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Mycophenolic acid (MPA), a common component of triple maintenance immunosuppressive regimens after organ transplantation, is administered as the 2-morpholinoethyl ester prodrug, mycophenolate mofetil (MMF, Cellcept®, Roche Laboratories, Inc., Nutley, NJ) to increase oral bioavailability.¹ The absorption of MMF from the gastrointestinal tract is >90%. In blood, MMF is rapidly and completely hydrolyzed to MPA and the MPA maximum concentration (Cmax) is achieved within 1 hour.² In plasma, MPA is highly bound to albumin and the unbound or free fraction of MPA (fMPA) is approximately 1% to 3% of the total concentration.³

Mycophenolic acid is subsequently glucuronidated in the liver to an inactive metabolite, phenyl mycophenolic acid glucuronide (MPAG), and a pharmacologically active metabolite, acyl glucuronide (Acyl-MPAG, AcMPAG).⁴ MPAG is then excreted in the bile and reabsorbed from the gastrointestinal tract, a process that creates a secondary peak in the plasma concentration-time profile.⁵ Renal excretion of MPAG is the major pathway for MPA elimination, whereas only 6% of MPA is excreted in the feces in an unchanged form, making renal function an important determinant of MPA plasma concentrations.⁵

Diabetes mellitus is a major cause of renal failure. Nearly 30% of renal allograft recipients in the United States have pre-existing type 1 or type 2 diabetes,⁶ and many also develop diabetes posttransplant.⁷ Diabetes is known to influence both the pharmacokinetics and pharmacodynamics of drugs.⁸ Diabetes-related delayed gastric emptying and gastroparesis may alter drug absorption and bioavailability. Diabetes also alters the biotransformation and metabolism of drugs by affecting the amount or activity of metabolizing enzymes,⁸ including those responsible for glucuronidation of drugs.⁹ Moreover, protein binding to albumin¹⁰ is altered, producing higher unbound plasma fractions and may increase clearance of drugs, such as MPA.

In an early report, Zanker et al¹¹ observed that the mean trough concentration of MPA was 1.24 mg/L in severely diabetic kidney-pancreas transplant recipients, significantly lower than the 2.94 mg/L measured in nondiabetic patients. Although 2 more recently published studies have described the concentration-time profile of total MPA in diabetic kidney-transplant recipients,^{12,13} neither of the 2 studies was specifically designed to address this question and also the differences in the concentrations of unbound MPA or MPA metabolites were not evaluated. The present investigation will characterize and compare the absorption, distribution, and elimination of MMF at steady-state in diabetic kidney-transplant recipients and matched nondiabetic patients.

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MATERIALS AND METHODS

The protocol was reviewed and approved by the institutional review board at Rhode Island Hospital (IRB #0159-03), and all patients gave informed consent to participate. Included were male and female patients of any race, ages 18 to 65 (inclusive) years who were at least 1 month postrenal allograft transplant and receiving triple immunosuppressive therapy that included MMF, a calcineurin inhibitor, and prednisone. Patients with previous documented diabetes mellitus, and patients with no evidence of diabetes were enrolled. Presence of diabetes mellitus was confirmed by patient histories, laboratory studies documenting glucose intolerance, and the need for oral hypoglycemic agents or exogenous insulin. Those with severe clinical gastroparesis defined by past hospitalization for intractable nausea and vomiting, uncontrolled diabetes as judged by a glycated hemoglobin (hemoglobin A_{1c}) level >9.5%, and evidence of severe liver disease, including abnormal liver enzyme levels or a total bilirubin level >3 times the upper limit of normal were excluded. Patients who were pregnant, nursing, or had received kidney/pancreas transplants also were not eligible for enrollment. The following criteria were used to match diabetic patients with the nondiabetic controls: gender, age \pm 10 years, race, daily dose of MMF, renal function, and type of donor (living or cadaveric). Patients also were matched based on the coadministration of cyclosporine or tacrolimus so that each diabetic patient on cyclosporine or tacrolimus would have a nondiabetic control on the same calcineurin inhibitor, therefore, balancing the impact of calcineurin inhibitor agent on the pharmacokinetics of MPA.

Pharmacokinetic Assessments

Patients were asked to fast overnight but clear liquids were allowed. On a typical study day, patients arrived at the center at 7:00 AM, underwent a physical examination, which included blood pressure, weight, height, and urinalysis assessments. Iohexol clearance was used to estimate glomerular filtration rate (GFR)¹⁴ and an acetaminophen absorption¹⁵ test was used to assess gastric emptying. Polyethylene catheters were inserted into a cubital vein in each arm: 1 for iohexol injection, and 1 for blood sample collection. A baseline blood sample was obtained before the administration of 10 mL of iohexol solution (Omnipaque 300; 647 mg of iohexol per mL corresponding to 300 mg of iodine per mL; Amersham Health Inc, Princeton, NJ) during 2 minutes. Normal saline 10 mL was injected to flush the catheter. At 8:00 AM, patients were administered their morning dose of immunosuppressive agents and other medications plus 1000 mg of liquid acetaminophen (Concentrated Tylenol® Infants' Drops, McNeil Consumer and Specialty Pharmaceuticals, Washington, PA) with 250 mL of water. Patients continued to fast for 2 hours but were allowed a fruit juice-based nutritional drink (Boost Breeze®, Novartis, Basel, Switzerland) to prevent hypoglycemia in diabetic patients. Blood was drawn from the contralateral vein into tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) at approximately 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 7, 9, 10, and 12 hours postdose. The blood samples were centrifuged for 10 minutes at 1500 \times g at room temperature and the separated plasma was stored at -70°C until analysis. Patients were encouraged to drink water

throughout the study and were served standard diabetic meals at 10:00 AM, and 1:00 PM, and 7:00 PM comprising a total of 2000 kcal per day.

Bioanalytics

All assays were performed at the Clinical Pharmacokinetics Research Laboratory, University of Rhode Island.

Total Plasma MPA, MPAG, and AcMPAG Concentrations

Concentrations of total MPA, MPAG, and AcMPAG were measured using a modified version of a previously published high performance liquid chromatography (HPLC) assay.¹⁶ The assay was modified to enable determination of AcMPAG in addition to MPA and MPAG in a single chromatographic run and was revalidated according to the guidance document for validation of analytical methods published by the United States Food and Drug Administration.¹⁷ In brief, the assay uses 2 internal standards: phenolphthalein glucuronic acid for MPAG and a carboxy butoxy derivative of MPA (MPAC) for AcMPAG and MPA. The method comprises a simple extraction procedure consisting of solid phase extraction using Isololute C₂ cartridges and analysis over a Zorbax Rx C₈ column (150 \times 4.6 mm, 5 μm) at 254 nm. The analytes were separated with gradient mixture of methanol and 0.1% phosphoric acid during a runtime of 14 minutes at a flow rate of 1 mL/min. The assay was linear in the concentration range from 0.2 to 50 mg/L for MPA, 0.5 to 25 mg/L for AcMPAG, and 2 to 500 mg/L for MPAG. The lower limit of quantification for MPA, AcMPAG, and MPAG were 0.2, 0.5 and 2 mg/L respectively. The mean \pm standard deviation interday accuracy and %CV for MPA was 100.3 \pm 5.7 and 5.7%, for AcMPAG was 102.6 \pm 5.7 and 5.6%, and MPAG was 100.5 \pm 5.3 and 5.3%.

Unbound Plasma MPA (fMPA) Concentrations

The concentration of plasma fMPA was measured using an ultrafiltration method followed by the analysis of MPA concentration in the plasma ultrafiltrate using liquid chromatography–tandem mass spectroscopy.¹⁶ An API 2000 mass spectrometer (MDS Sciex, Toronto, Canada) was used for all quantification. The assay was linear in the concentration range of 1 to 1000 $\mu\text{g/L}$ for fMPA with a lower limit of quantification of 1 $\mu\text{g/L}$ and an accuracy of >95%.

Acetaminophen Plasma Concentrations

Concentrations were measured by a validated HPLC assay adapted from Brunner and Bai.¹⁸ In brief, 600 μL of protein precipitant solution (perchloric acid in water, 6%, vol/vol) containing 50 $\mu\text{g/mL}$ of the internal standard theophylline was added to 300 μL of patient plasma. The samples were vortex mixed and centrifuged at 15,000 \times g for 10 minutes and 30 μL of the supernatant was injected into HPLC (Hitachi D-7000 series instrument, San Jose, CA) consisting of an autosampler fitted with a 200 μL sample loop, a quaternary pump, a column oven, and a variable wavelength UV detector set at 254 nm. The analytical column used was a Supelcosil LC-18, 150 \times 4.6 mm, 5 μm particle size, maintained at 30°C. The mobile phase consisted 0.05 mM Na₂SO₄ buffer (pH = 2.2)

and 5% vol/vol acetonitrile. The assay was linear over a concentration range of 1 to 200 mg/L. The limit of quantification was 1 mg/L and the accuracy of the method was >92%.

Iohexol Plasma Concentrations

The plasma concentration of iohexol was quantified using a recently developed and published HPLC method¹⁴ that is accurate at iohexol concentrations ranging from 10 to 750 mg/L. The method had an accuracy of >92% and intraday and interday coefficient of variation (CV) of <3.7% and <3.6%, respectively.

Glycated Albumin Concentrations

Plasma glycated albumin content was measured using an enzyme-linked immunosorbent assay (ELISA) (Glycaben[®], Exocell, Inc., Philadelphia, PA). Glycated albumin measurements provide information about glycemic control analogous to that offered by glycohemoglobin measurements except that the protein measured is glycated albumin, which has a shorter half-life than hemoglobin. This test was performed to verify the degree of glycation of albumin, the major binding protein for MPA.

Pharmacokinetic Calculations

All pharmacokinetic calculations were performed using noncompartmental and/or compartmental analyses implemented in WinNonlin[®] version 4.1 (Pharsight Inc., Mountain View, CA). Actual sampling times were used for all analysis. Acetaminophen-related pharmacokinetic parameters were calculated based on plasma samples obtained through 5 hours postdose. GFR values were estimated by calculating the iohexol clearance from iohexol plasma concentrations measured predose through 7 hours postdose using a noncompartmental analysis, followed by normalizing the iohexol clearance by a body surface area of 1.73 m².

For MPA, MPAG, AcMPAG, fMPA concentration-time data, area under the concentration-time curve from time zero to the last sampling time (AUC₀₋₁₂) were calculated using linear trapezoidal rule. The minimum concentration (C_{min}), the maximum concentration (C_{max}), and the time to the maximum concentration (T_{max}) were calculated for all four analytes. Apparent oral clearance of MPA was calculated by dividing the MPA content of the MMF dose by the AUC₀₋₁₂.¹⁹ The values of MPA AUC also were expressed as dose-normalized values to 1000 mg of MMF. Because MPA undergoes enterohepatic recirculation, no attempt was made to estimate elimination rate constant, half-life or AUC_{0-infinity}. Mean residence time (MRT) is the average amount of time a drug molecule remains in the body and provides a good approximation of half-life. The MRT for MPA was calculated by dividing the values of area-under-the-moment-curve (AUMC) by the AUC.

Unbound fraction of MPA was calculated by dividing fMPA by total MPA concentrations and is expressed as a percentage. The parent-to-metabolite concentration ratios (ie, MPA/MPAG or MPA/AcMPAG concentration ratios) were calculated by dividing the reported MPA concentrations by the metabolite concentration that was corrected for the differences in the MPA and glucuronide molecular weights

(320.35/539.42). As the molecular weight of MPAG and AcMPAG are identical, no such correction was applied to the calculation of MPAG to AcMPAG ratio.

Data Analysis

All statistical analyses were performed using the SPSS software (Version 10.1, SPSS Inc., Chicago, IL). All data were subjected to Kolmogorov-Smirnov test to verify their normal distribution, reported as mean ± standard deviation and analyzed by using a parametric statistical test. Unless otherwise stated, all nonnormally distributed data were transformed to its natural logarithmic base and analyzed. If the log transformation did not resolve the non-Gaussian distribution, data were reported as median and interquartile range (25% and 75% intervals) and were analyzed using appropriate non-parametric test.

RESULTS

The patient population included 24, stable, kidney-transplant recipients, 13 of whom had diabetes mellitus. Among the patients with diabetes, 6 had type 1 diabetes, 5 had type 2, and 2 had developed diabetes after transplantation. The etiology of end-stage renal disease in these patients was diabetic nephropathy (n = 11), IgA nephropathy (n = 5), polycystic kidney disease (n = 3), crescentic glomerulonephritis (n = 1), focal segmental glomerulosclerosis (n = 1), hypertension (n = 1), hypertensive nephrosclerosis (n = 1), and membranous glomerulonephritis (n = 1). In general, matching of diabetic and nondiabetic kidney transplant recipients ensured comparable demographics and other characteristics between the 2 groups (Table 1). Also daily dose of prednisone was the same between the 2 groups. As expected, both serum glucose concentrations and the percentage of hemoglobin A_{1c} were significantly higher in patients with diabetes.

The existence of delayed gastric emptying was confirmed by the results of the acetaminophen absorption test (Fig. 1). The C_{max} of acetaminophen was lower (11.42 ± 3.64 vs. 15.21 ± 3.36 mg/L; *P* = 0.02) and the T_{max} was longer (76.1 ± 49.8 vs. 29.8 ± 11.6 minutes; *P* = 0.009) in patients with diabetes versus those without diabetes. The extent of acetaminophen absorption (AUC₀₋₅) was not significantly different (*P* = 0.79) between the 2 groups.

The pharmacokinetic characteristics of total MPA are presented in Table 2 and Figure 2A. In summary, all pharmacokinetic parameters, including MPA C_{max}, AUC₀₋₁₂, dose normalized AUC₀₋₁₂, apparent oral clearance, and mean residence time were comparable between the 2 groups with the exception of T_{max} (*P* = 0.04). In addition, with the exception of T_{max}, none of the pharmacokinetic parameters differed between patients with and without diabetes when data were stratified by calcineurin inhibitor, cyclosporine, or tacrolimus. The concentration-time profiles of total MPA in 17 of 24 patients showed signs of enterohepatic recirculation characterized by a secondary peak in plasma concentration-time data. Of these profiles 11 diabetic patients (84%) showed signs of enterohepatic recirculation as compared with 6 nondiabetic patients (54%) (*P* = 0.18). The morning and afternoon trough concentrations of MPA was slightly but not significantly higher

TABLE 1. Patient Demographics and Other Characteristics

	Diabetic Patients (n = 3)	Nondiabetic Patients (n = 11)
Male gender	12	10
Time posttransplant (days)	1047 ± 762	860 ± 549
Age (yr)	55 ± 11	53 ± 11
Weight (kg)	87 ± 22	85 ± 16
Ethnic origin		
White	12	10
African American	1	1
Serum creatinine (g/dL)	1.43 ± 0.3	1.36 ± 0.43
Creatinine clearance (mL/min)*	72 ± 20	82 ± 38
Serum glucose (g/L)	149.9 ± 86.7	91.2 ± 24.2‡
Hemoglobin A _{1c} (%)	7.8 ± 1.6	5.6 ± 1§
Mycophenolate mofetil morning dose (mg)	654 ± 240	659 ± 280
Cyclosporine/tacrolimus coadministration	6/7	6/5
Cyclosporine/tacrolimus daily dose (mg)	158 ± 138/ 4.4 ± 2.1	71 ± 25/ 10.6 ± 6
Cyclosporine/tacrolimus trough concentration (μg/L)†	77.4 ± 28.1/ 9.6 ± 3.7	113.8 ± 62.2/ 12.4 ± 4.7
Prednisone daily dose (mg)	6.4 ± 3.2	6.4 ± 2.9

Data are numbers or means ± standard deviations.

*Estimated by using Cockcroft and Gault equation.

†Trough concentration of cyclosporine and tacrolimus were measured using LC/MS/MS.

‡P < 0.05; §P < 0.001.

TABLE 2. Comparison of the Pharmacokinetic Profile of Mycophenolic Acid in Diabetic and Nondiabetic Patients

	Diabetic Patients (n = 13)	Nondiabetic Patients (n = 11)	P
MPA Tmax (min)	86.4 ± 41.4	52.8 ± 31.8	0.04
MPA morning trough levels (mg/L)	2.67 ± 3.96	1.67 ± 1.36	0.44
MPA evening trough levels (mg/L)	2.32 ± 2.52	1.67 ± 1.33	0.45
MPA Cmax (mg/L)	11.72 ± 10.28	11.12 ± 8.63	0.87
MPA AUC ₀₋₁₂ (mg*hr/L)	46.72 ± 45.52	35.24 ± 17.92	0.44
MPA dose-normalized AUC (mg*hr/L)*	66.45 ± 44.26	55.74 ± 25.26	0.48
Apparent clearance (L/hr)	15.47 ± 8.6	15.87 ± 7.57	0.9
Mean residence time (hr)	4.6 ± 0.66	4.20 ± 0.7	0.16

MPA, mycophenolic acid; AUC₀₋₁₂, area under the concentration-time curve from 0 to 12 hours; Cmax, maximum plasma concentration; Tmax, time to maximum plasma concentration.

Data are means ± standard deviations except for P values.

*Dose-normalized AUC values are normalized to 1000 mg of mycophenolate mofetil.

in diabetic patients than nondiabetics (Table 2). This could be because the concentration-time profile from a larger portion of diabetic patients showed signs of enterohepatic recirculation.

The pharmacokinetic properties of unbound MPA are shown in Table 3 and Figure 2B. Only the time to reach the maximum fMPA concentration (fMPA Tmax) was significantly different (P = 0.04) between the 2 groups, which is an indication of delayed MPA absorption in diabetic patients. The

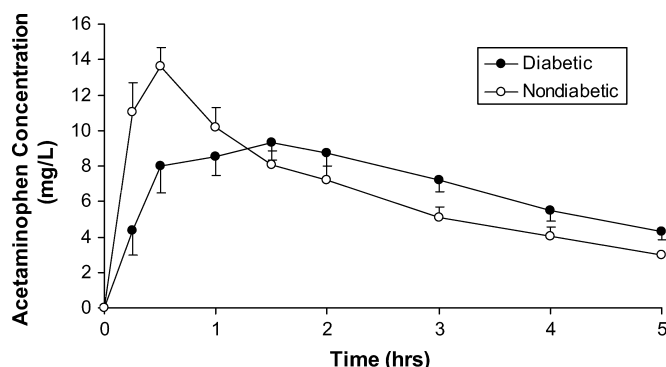


FIGURE 1. The average concentration-time profile of acetaminophen in diabetic and nondiabetic kidney-transplant recipients (in diabetic patients the acetaminophen concentrations were significantly lower at 15 and 30 minutes postdose; P < 0.02, error bars represent standard error of the mean).

unbound fraction of MPA, which is the indicator of extent of plasma protein binding, was not different (Table 3). In addition, neither serum albumin concentration nor the percentage of glycated albumin, an indicator of glucose control in the preceding 3 weeks, was significantly different between the 2 groups.

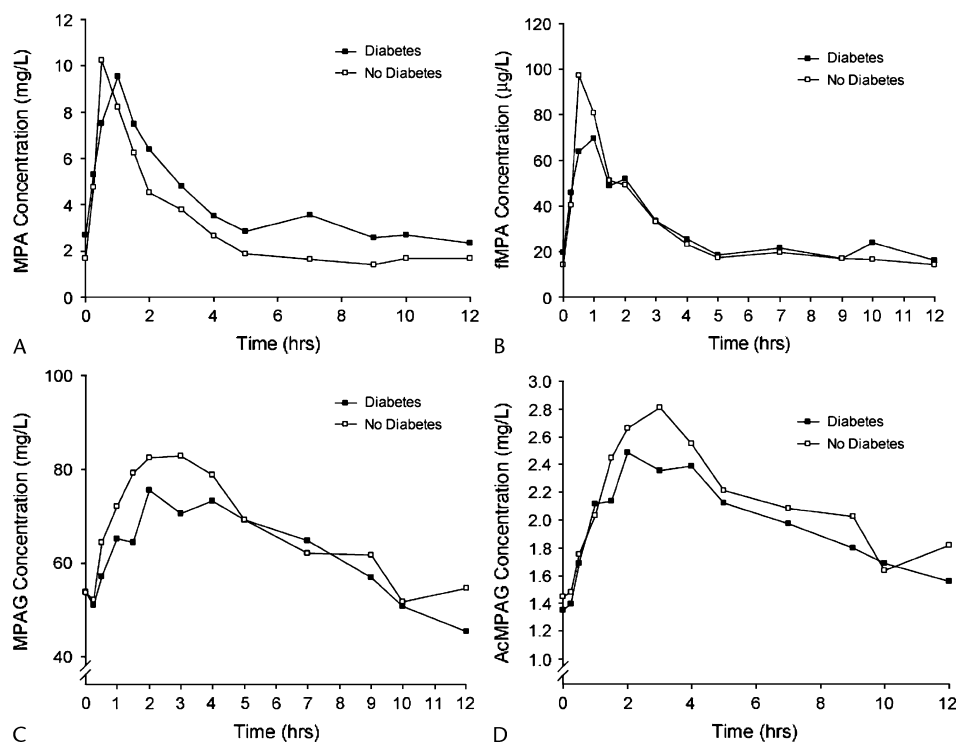
Table 4 and Figures 2C, D present the pharmacokinetic characteristics of the MPA metabolites in both groups of patients. The Cmin, Cmax, and AUC₀₋₁₂ of both metabolites, MPAG and AcMPAG, were similar in the 2 patient groups. However, the ratio of inactive to active metabolites, MPAG to AcMPAG, was significantly higher in patients with diabetes (P = 0.03).

Although, on average, patients with diabetes had a 12 mL/min lower estimated GFR, measured using an iohexol clearance method, than patients without diabetes, GFR values were not significantly different between the 2 study groups (P = 0.12). Figures 3A–C depicts the association between GFR and dose normalized area under the concentration-time curves (AUC₀₋₁₂) of MPA, MPAG, and AcMPAG. The Pearson correlation coefficients were 0.028 (P = 0.9) for MPA AUC and GFR, -0.547 (P = 0.006) for MPAG AUC and GFR and -0.313 (P = 0.137) for AcMPAG AUC and GFR, and these values were comparable between diabetic and nondiabetic patients.

DISCUSSION

The present study was designed to evaluate the impact of type 1 or type 2 diabetes mellitus on the pharmacokinetics of the immunosuppressant MMF in stable kidney-transplant recipients. To this aim, the absorption, distribution, plasma protein binding, metabolism, and elimination of MMF were compared in kidney transplant recipients with and without diabetes. Rigorous inclusion, exclusion, and matching criteria were applied to minimize demographic and physiologic

FIGURE 2. A, The concentration-time profile of mycophenolic acid (MPA) in diabetic and nondiabetic kidney-transplant recipients (all concentrations are expressed as dose-normalized values to 1000 mg of mycophenolate mofetil). B, The concentration-time profile of unbound mycophenolic acid (fMPA) in diabetic and nondiabetic kidney-transplant recipients (all concentrations are expressed as dose-normalized values to 1000 mg of mycophenolate mofetil). C, The concentration-time profile of mycophenolic acid glucuronide (MPAG) in diabetic and nondiabetic kidney-transplant recipients (all concentrations are expressed as dose-normalized values to 1000 mg of mycophenolate mofetil). D, The concentration-time profile of mycophenolic acid acyl-glucuronide (AcMPAG) in diabetic and nondiabetic kidney-transplant recipients (all concentrations are expressed as dose-normalized values to 1000 mg of mycophenolate mofetil).



variability between patient groups. This strategy has ensured a well-matched, case-control investigation as evidenced by the lack of significant differences in the demographic characteristics of the patients.

The only significant difference in MPA pharmacokinetics found in this study, between patient groups, was the delay in the rate of absorption of MPA in patients with diabetes as demonstrated by MPA and fMPA T_{max} values. There was no significant reduction in the extent of absorption. This study did exclude patients with severe clinical gastroparesis. The acetaminophen absorption test, is an easily implemented, well-established method for evaluating gastric emptying that has a high correlation with gastric scintigraphy.²⁰ Acetaminophen is a basic compound that is exclusively absorbed after reaching the intestine.¹⁵ Using this method, we have confirmed that diabetes-related delayed gastric emptying most likely slowed down the MMF absorption.

Because MPA is highly bound to albumin, the extent of plasma protein binding and the concentration of fMPA have important implications with respect to its pharmacokinetic properties and pharmacodynamic effects. The concentration of fMPA also is believed to be associated with the occurrence of MPA-related side effects.²¹ At therapeutic plasma concentrations, MPA binds to albumin in a linear fashion; the plasma concentration and binding capacity of albumin, in turn, affects the extent of bound MPA and fMPA in plasma. Hyperglycemia modifies the microenvironmental condition of the type 2 binding site of albumin and consequently reduces its binding capacity.^{10,22,23} Thus, in the setting of diabetes, the extent of protein binding of numerous drugs can be altered.¹⁰ In the present study, we rigorously evaluated fMPA plasma concentrations, by taking measurements at 12 occasions during the

dosing interval.¹⁶ The liquid chromatography-mass spectrometry method was optimized to resolve MPAG and AcMPAG peaks from MPA chromatographically, because both metabolites can break down into MPA at the mass spectrometer ion source and cause erroneous results for fMPA concentration measurements.¹⁶ No differences were observed between the extent of plasma protein binding as characterized by fMPA unbound fraction or exposure to fMPA as characterized by fMPA AUC_{0-12} . It must be noted that patients with uncontrolled hyperglycemia were excluded from this study. In

TABLE 3. Comparison of the Pharmacokinetic Profile of Unbound Mycophenolic Acid in Diabetic and Nondiabetic Patients

	Diabetic Patients (n = 13)	Nondiabetic Patients (n = 11)	P
fMPA T_{max} (min)	97.3 ± 54.3	59.5 ± 29.3	0.04
fMPA morning trough (µg/L)	19.52 ± 18.51	14.37 ± 11.22	0.42
fMPA afternoon trough (µg/L)	16.02 ± 10.66	14.42 ± 10.12	0.71
fMPA C_{max} (µg/L)	93.7 ± 71.7	115.3 ± 111.6	0.57
fMPA AUC_{0-12} (µg*hr/L)	343.7 ± 191	332.4 ± 247.7	0.9
MPA fraction unbound (%)	0.9 (0.63–1.37)	0.95 (0.54–1.52)	0.84
Serum albumin concentration (g/dL)	4.14 ± 0.34	4.33 ± 0.19	0.12
Glycated albumin (%)	1.32 ± 0.28	1.17 ± 0.38	0.3

fMPA, unbound mycophenolic acid; AUC_{0-12} , area under the concentration-time curve from 0 to 12 hours; C_{max} , maximum plasma concentration; T_{max} , time to maximum plasma concentration.

Data are means ± standard deviations or medians (interquartile ranges) except for P values.

TABLE 4. Comparison of the Estimated Glomerular Filtration Rate and the Pharmacokinetic Profile of Mycophenolic Acid Metabolites in Diabetic and Nondiabetic Patients

	Diabetic Patients (n = 13)	Nondiabetic Patients (n = 11)	P
Glomerular filtration rate (mL/min/1.73 m ²)	39.8 ± 17.9	52.7 ± 21.4	0.12
MPAG			
Cmin (mg/L)	40.7 ± 20	47.5 ± 30.8	0.52
Cmax (mg/L)	84.0 ± 46.7	87 ± 50.5	0.88
AUC ₀₋₁₂ (mg*hr/L)	737 ± 353	810 ± 479	0.67
AcMPAG			
Cmin (mg/L)	1.17 ± 0.56	1.33 ± 0.74	0.57
Cmax (mg/L)	2.91 ± 1.81	2.95 ± 1.75	0.96
AUC ₀₋₁₂ (mg*hr/L)	23.6 ± 13.3	26.2 ± 15.6	0.67
MPAG to AcMPAG concentration ratio	34.1 (25.1–42.2)	29.2 (24.6–36.1)	0.03
MPA to MPAG concentration ratio	0.08 (0.03–0.16)	0.08 (0.04–0.16)	0.48
MPA to AcMPAG concentration ratio	2.66 (1.33–5.33)	2.4 (1.2–4.22)	0.24

AcMPAG, mycophenolic acid acyl glucuronide; AUC₀₋₁₂, area under the concentration-time curve from 0 to 12 hours; Cmax, maximum plasma concentration; Cmin, minimum plasma concentration; MPAG, mycophenolic acid glucuronide.

Data are means ± standard deviations or medians (interquartile ranges) except for P values.

addition, concentrations of glycated albumin, an indicator of glycemic control 3 weeks before the assessment, was not different between the 2 groups, further substantiating that plasma protein binding of MPA was not affected in these patients in whom blood glucose levels were controlled. It would be of interest to investigate the plasma protein binding of MPA in poorly controlled diabetic patients in whom the percentage of glycated albumin is significantly elevated.

MPAG, the inactive metabolite of MPA, undergoes enterohepatic recirculation and is largely renally excreted. The pharmacologically active metabolite, AcMPAG, is considered responsible for MPA-related adverse effects. Acyl migration

has been reported to induce the production of reactive metabolites that bind to cellular and serum proteins and the development of immunotoxicity.⁴ Both of these metabolites are produced by the enzymatic activity of various UDP-glucuronosyltransferase (UGT) isoforms. Among them, UGT1A9 and UGT2B7 seem to play a major role in the metabolism of MPA to MPAG and AcMPAG respectively.²⁴ In animal models, diabetes has been shown to induce changes in the metabolism of endogenous or exogenous substances. For example, in streptozotocin-induced diabetic rats, Watkins and Sherman⁹ observed that the hepatobiliary clearance of acetaminophen and digoxin, both UGT substrates, was altered, whereas the clearance of bilirubin remained unchanged. In spontaneously diabetic rats, Braun and associates²⁵ demonstrated an increased transcription of the UGT1A1 enzymatic system. In diabetic patients, however, only few studies indicate a change in amount or activity of enzymes involved in drug metabolism.^{8,10}

No significant change in MMF metabolism was observed in this study nor did the pharmacokinetics of MPAG and AcMPAG significantly differ between patients with and without diabetes. Also splitting the data into subgroups based on coadministration of cyclosporine or tacrolimus did not produce any significant differences in the MPA metabolites between diabetic and nondiabetic patients. Indeed Cmax, Cmin, and AUC values for MPA metabolites were similar in both groups. There is ample evidence for a drug-drug interaction between cyclosporine and MPA. It is believed that cyclosporine inhibits the MRP2 (ABCC2) transporter and, therefore, decreases the enterohepatic recirculation of MPA,²⁶ hence, cyclosporine-treated patients are administered MPA at higher doses than those taking tacrolimus.²⁷ Only the ratio of MPAG to AcMPAG was significantly higher in diabetic kidney recipients, indicating that less AcMPAG was formed or more excreted. The difference in the ratio of MPAG to AcMPAG could not be explained by differences in the kidney function, because GFR values between the 2 groups were not significantly different and AcMPAG AUC does not seem to be related to GFR. To better understand this finding, further investigation using in vitro drug metabolism methods will be required.

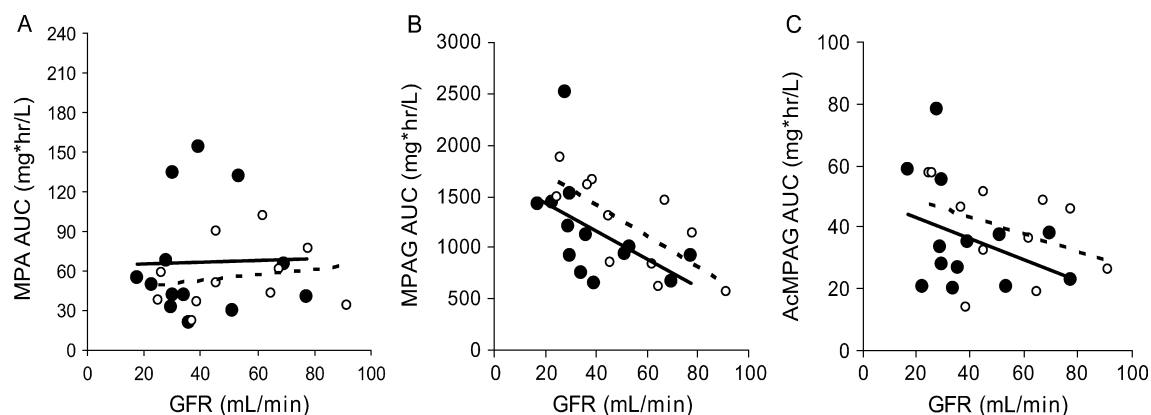


FIGURE 3. Association between glomerular filtration rate (GFR) and the Area Under the Concentration-Time curve (AUC₀₋₁₂) for MPA (A), MPAG (B), and AcMPAG (C). (diabetic: closed circles, solid line; nondiabetic: open circles, dashed line).

AU1

This study was designed to include approximately equal numbers of diabetic and nondiabetic patients taking cyclosporine or tacrolimus. In this way, we could evaluate the impact of diabetes on the pharmacokinetics of MPA in patients under both types of calcineurin inhibitor treatment. Also the dose of oral prednisone was comparable between the 2 groups. Splitting the data into subgroups based on the calcineurin inhibitor coadministration did not produce any significant differences in any of the pharmacokinetic parameters, including the MPA metabolites between diabetic and nondiabetic patients.

In conclusion, we have compared, in a case-controlled manner, the concentration-time profiles of MPA, fMPA, and 2 major metabolites, MPAG and AcMPAG, in stable renal-transplant recipients with and without diabetes. Except for an initial slower absorption rate, no significant difference was observed in the characteristics of MPA, the amount of fMPA, or of MPA metabolites between the 2 groups. In the context of clinical management of transplant recipients, the lack of difference in the exposure to MPA and fMPA between the 2 groups will be reassuring in the clinical management of transplant recipients with well-controlled diabetes mellitus. In the context of pharmacokinetic study design or implementation, because diabetic patients absorb MPA somewhat later than the other patients, additional sampling time points will be required during the first 2 to 3 hours after drug administration in these patients. It also would be interesting to evaluate the pharmacokinetics of MMF in diabetic patients who have poorly controlled diabetes and/or suffer from severe gastroparesis.

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REFERENCES

1. Sollinger HW for the U.S. renal transplant mycophenolate mofetil study group. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. *Transplantation*. 1995;60:225–232.
2. Bullingham R, Monroe S, Nicholls A, et al. pharmacokinetics and bioavailability of mycophenolate mofetil in healthy subjects after single-dose oral and intravenous administration. *J Clin Pharmacol*. 1996;36:315–324.
3. Nowak I, Shaw LM. Mycophenolic acid binding to human serum albumin: characterization and relation to pharmacodynamics. *Clin Chem*. 1995;41:1011–1017.
4. Shipkova M, Armstrong VW, Oellerich M, et al. Acyl glucuronide drug metabolites: toxicological and analytical implications. *Ther Drug Monit*. 2003;25:1–16.
5. Bullingham RE, Nicholls AJ, Kamm BR. Clinical pharmacokinetics of mycophenolate mofetil. *Clin Pharmacokinet*. 1998;34:429–455.
6. Wynn JJ, Distant DA, Pirsch JD, et al. Kidney and pancreas transplantation. *Am J Transplant*. 2004;4(Suppl 9):72–80.

7. Kasiske BL, Snyder JJ, Gilbertson D, et al. Diabetes mellitus after kidney transplantation in the United States. *Am J Transplant*. 2003;3:178–185.
8. Gwilt PR, Nahhas RR, Tracewell WG. The effects of diabetes mellitus on pharmacokinetics and pharmacodynamics in humans. *Clin Pharmacokinet*. 1991;20:477–490.
9. Watkins JB III, Sherman SE. Long-term diabetes alters the hepatobiliary clearance of acetaminophen, bilirubin and digoxin. *J Pharmacol Exp Ther*. 1992;260:1337–1343.
10. Zini R, Riant P, Barre J, et al. Disease-induced variations in plasma protein levels, implications for drug dosage regimens (part I). *Clin Pharmacokinet*. 1990;19:147–159.
11. Zanker B, Sohr B, Eder M, et al. Comparison of MPA trough levels in patients with severe diabetes mellitus and from non-diabetics after transplantation. *Transplant Proc*. 1999;31:1167.
12. Pescovitz MD, Guasch A, Gaston R, et al. Equivalent pharmacokinetics of mycophenolate mofetil in African-American and Caucasian male and female stable renal allograft recipients. *Am J Transplant*. 2003;3:1581–1586.
13. van Hest RM, Mathot RA, Vulto AG, et al. Mycophenolic acid in diabetic renal transplant recipients: pharmacokinetics and application of a limited sampling strategy. *Ther Drug Monit*. 2004;26:620–625.
14. Soman RS, Zahir H, Akhlaghi F. Development and validation of an HPLC-UV method for determination of iohexol in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2005;816:339–343.
15. Willems M, Quartero AO, Numans ME. How useful is paracetamol absorption as a marker of gastric emptying? A systematic literature study. *Dig Dis Sci*. 2001;46:2256–2262.
16. Patel CG, Mendonza AE, Akhlaghi F, et al. Determination of total mycophenolic acid and its glucuronide metabolite using liquid chromatography with ultraviolet detection and unbound mycophenolic acid using tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2004;813:287–294.
17. Center for Drug Evaluation and Research. Food and Drug Administration. Bioanalytical Method Validation; Guidance for Industry. Available at: <http://www.fda.gov/cder/guidance/4252fnl.htm>. Date accessed March 2005 2001.
18. Brunner LJ, Bai S. Simple and rapid assay for acetaminophen and conjugated metabolites in low-volume serum samples. *J Chromatogr B Biomed Sci Appl*. 1999;732:323–329.
19. Rowland M, Tozer TN. *Clinical Pharmacokinetics, Concepts and Applications*. 3rd ed. Philadelphia: Williams & Wilkins; 1995.
20. Kelly K, O'Mahony B, Lindsay B, et al. Comparison of the rates of disintegration, gastric emptying, and drug absorption following administration of a new and a conventional paracetamol formulation, using gamma scintigraphy. *Pharm Res*. 2003;20:1668–1673.
21. Kaplan B, Gruber SA, Nallamathou R, et al. Decreased protein binding of mycophenolic acid associated with leukopenia in a pancreas transplant recipient with renal failure. *Transplantation*. 1998;65:1127–1129.
22. Zini R, Riant P, Barre J, et al. Disease-induced variations in plasma protein levels, implications for drug dosage regimens (part II). *Clin Pharmacokinet*. 1990;19:218–229.
23. Okabe N, Hashizume N. Drug binding properties of glycosylated human serum albumin as measured by fluorescence and circular dichroism. *Biol Pharm Bull*. 1994;17:16–21.
24. Picard N, Ratanasavanh D, Premaud A, et al. Identification of the UDP-glucuronosyltransferase isoforms involved in mycophenolic acid phase II metabolism. *Drug Metab Dispos*. 2005;33:139–146.
25. Braun L, Coffey MJ, Puskas F, et al. Molecular basis of bilirubin UDP-glucuronosyltransferase induction in spontaneously diabetic rats, acetone-treated rats and starved rats. *Biochem J*. 1998;336:587–592.
26. Kobayashi M, Saitoh H, Kobayashi M, et al. Cyclosporin A, but not tacrolimus, inhibits the biliary excretion of mycophenolic acid glucuronide possibly mediated by Mrp2 in rats. *J Pharmacol Exp Ther*. 2004;309:1029–1035.
27. Shaw LM, Korecka M, Venkataramanan R, et al. Mycophenolic acid pharmacodynamics and pharmacokinetics provide a basis for rational monitoring strategies. *Am J Transplant*. 2003;3:534–542.

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