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Effect of Diabetes Mellitus on Mycophenolate Sodium Pharmacokinetics and Inosine Monophosphate Dehydrogenase Activity in Stable Kidney Transplant Recipients

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Abstract

Effect of diabetes mellitus on mycophenolic acid (MPA) pharmacokinetics and catalytic activity of inosine monophosphate dehydrogenase (IMPDH) was investigated in maintenance kidney transplant recipients. Demographically matched diabetic (n = 9) and nondiabetic (n = 9) patients were included in a 12-hour open-label, steady-state study after oral administration of enteric-coated mycophenolate sodium. Concentrations of total MPA and free MPA, MPA-glucuronide, and acyl-MPA-glucuronide were measured and oral acetaminophen absorption was used as a marker for gastric-emptying rate. Median (range) of MPA area under the curve_{0–12} was 36.7 (range, 16.4–116.4) mg^{*}h/L in diabetic and 48.2 (range, 34.9–80.1) mg^{*}h/L in nondiabetic patients (*P* = 0.49). All other primary pharmacokinetic parameters, including time to maximum concentration, for total or unbound MPA as well as MPA metabolites were comparable. In contrast, IMPDH activity was 17.5 ± 2.8 versus 46.6 ± 2.5 nmol XMP/h/mg protein in diabetics and nondiabetics, respectively (*P* < 0.0001) and was significantly lower in the diabetics irrespective of concomitant therapy with cyclosporine or tacrolimus. This study demonstrated that diabetes does not alter MPA pharmacokinetics when administered as enteric-coated mycophenolate sodium; however, IMPDH activity appeared to be significantly lower in patients with diabetes independent of the unbound or total concentrations of MPA. Further investigations are warranted to investigate the regulation of IMPDH enzyme in patients with diabetes.

Keywords

diabetes mellitus; IMPDH activity; kidney transplantation; mycophenolic acid; pharmacokinetics

INTRODUCTION

Approximately half of all kidney transplant recipients in the United States have diabetes mellitus. A recent analysis of approximately 30,000 kidney transplant recipients included in the U.S. Renal Data System showed that 42% of patients had pretransplant diabetes.¹ Moreover, 15% to 20% of patients develop diabetes after transplantation, a condition that is commonly known as posttransplant diabetes mellitus.^{1–3} Diabetes can influence the pharmacokinetic characteristics of drugs by affecting drug absorption (as a result of gastroparesis or delayed gastric emptying), protein binding (as a result of glycation of albumin), and metabolism (because of differential regulation of drug-metabolizing enzymes by diabetes and associated conditions).^{4,5}

We have previously evaluated the pharmacokinetics of mycophenolic acid (MPA) and its metabolites when administered as mycophenolate mofetil (MMF; Cellcept; Roche Laboratories, Nutley, NJ).⁶ It was observed that diabetes did not significantly alter MPA exposure, but patients with diabetes exhibited a pronounced delay in the absorption of MPA because of increased gastric-emptying time.⁶ Similarly, the rate of absorption of MPA from MMF formulation was lower in nondiabetic patients with delayed gastric emptying.⁷

The recently-approved Myfortic (Novartis Pharmaceuticals, East Hanover, NJ) is an enteric-coated formulation of the sodium salt derivative of mycophenolic acid (EC-MPS). The peak plasma concentration of MPA with EC-MPS occurs 2.5 to 3 hours after oral dosing,⁸ almost 1.5 to 2 hours later than with MMF. It is conceivable that the delay in absorption associated with EC-MPS may be amplified in patients with diabetes as a result of gastroparesis. A post hoc analysis of data from clinical trials converting kidney transplant recipients from a MMF to an EC-MPS regimen showed that EC-MPS is safe and effective in patients with diabetes; however, the pharmacokinetics of EC-MPS in patients with diabetes has never been characterized.⁹

Diabetic kidney transplant recipients are generally more susceptible to infection because of reduced innate immunity,^{1,10–12} but the effect of diabetes on the molecular target for MPA [inosine 5'-monophosphate dehydrogenase (IMPDH)] is not known. Because kidney transplant recipients are maintained on large daily doses of immunosuppressive agents, it is important to determine whether immunologic differences exist between diabetic and nondiabetic patients so the immunosuppressive regimen can be tailored to the individual needs of each patient.

The current study had two objectives; first, to investigate the influence of diabetes on the concentration–time profiles of mycophenolic acid and metabolites, MPA-glucuronide (MPAG) and acyl-MPAG (AcMPAG) when administered as EC-MPS; and second, to compare the catalytic activity of the IMPDH, the molecular target for MPA, between maintenance diabetic and nondiabetic kidney transplant recipients.

MATERIALS AND METHODS

Study Design

This was a prospective, open-label study of MPA pharmacokinetics and pharmacodynamics in kidney transplant recipients with stable graft function undertaken over a 12-hour period. Institutional Review Board approval was obtained and all patients provided informed consent.

Patient Population and Immunosuppression

Recruitment was designed to include equal numbers of patients in the diabetic and nondiabetic groups. Patients with either controlled or uncontrolled (glycated hemoglobin greater than 9.5% and significant fluctuation in glucose level) diabetes were eligible for inclusion. Patients were excluded if they were younger than age 18 years, had undergone transplantation 3 months or less previously, or were pregnant or nursing. Transplant recipients with evidence of severe liver disease (aspartate transaminase or alanine transaminase level greater than three times the upper limit of normal) were also excluded. The following criteria were used to match patients with diabetes with nondiabetic control subjects: gender, age ± 10 years, race, calcineurin inhibitor: cyclosporine versus tacrolimus, time posttransplant groups (3–6 months, longer than 6 months), comparable renal function within ± 30 mL/min estimated creatinine clearance, and type of donor (living versus cadaver).

All eligible patients were receiving a triple immunosuppressive regimen consisting of MPA, cyclosporine or tacrolimus, and prednisone. At the time of recruitment, patients were receiving MMF but were converted to EC-MPS 2 weeks or longer before the day of the study. Blood samples from apparently healthy individuals ($n = 5$, age 25–40 years) were used as controls for determination of IMPDH catalytic activity. All patients were on a twice-daily EC-MPS regimen.

Sampling Procedures

On the day of the study, patients were required to report to the hospital at 7:00 AM, having fasted overnight, at which time they underwent physical examination with subsequent collection of baseline urine and blood samples. To avoid hypoglycemia, patients were given the option of having a clear fruit juice-based drink (Novartis Medical Nutrition, Fremont, MI) after administration of EC-MPS, but generally remained fasted for 2 hours after the study started. Patients were served three standard diabetic hospital meals consisting of 2000 kcal/day at 10:00 AM, 1:00 PM, and 7:00 PM. Typically, a pharmacokinetic study was started at 8:00 AM and lasted 12 hours.

Polyethylene catheters were inserted into the cubital vein in the arm and a predose blood sample was obtained. The patients then took their regular morning dose of immunosuppressants (EC-MPS, cyclosporine or tacrolimus, prednisone) with a 250-mL glass of water and medication prescribed for coexisting conditions, including hypoglycemic, antifungal, antihypertensive, and antihyperlipidemic medications. In addition, 1000 mg acetaminophen oral solution was given for the assessment of gastric emptying and 10 mL

iohexol (Omnipaque 300; 647 mg/mL; Amersham Health, GE Healthcare Inc., Princeton, NJ) was given intravenously for the assessment of glomerular filtration rate.⁶ Blood samples were collected in ethylene-diaminetetra-acetic acid tubes at predose, 0.25, 0.5, 1.0, 1.5, 2, 3, 5, 7, 9, 10, 11, and 12 hours after the EC-MPS dose was administered and the exact time of blood collection was recorded. Samples were immediately centrifuged at 1500 g and plasma was stored at -80°C until analysis. In addition, heparinized blood was collected predose and at 1, 2, 3, 7, and 12 hours postdose for the assessment of IMPDH activity.

Determination of Drug Concentrations

The concentration of total MPA, MPAG, and AcMPAG were measured using high-performance liquid chromatography– with ultraviolet detection (HPLC-UV).¹³ The concentration of free MPA was assessed using ultrafiltration followed by liquid chromatography/mass spectrometry/mass spectrometry in plasma.¹⁴ Concentrations of iohexol¹⁵ and acetaminophen⁶ were measured by HPLC-UV. All methods were developed in-house and validated according to the guidelines set by the U.S. Food and Drug Administration.¹⁶

Determination of Inosine Monophosphate Dehydrogenase Activity

IMPDH activity in peripheral blood mononuclear cells was measured based on a previously published assay¹⁷ with major modification to the chromatographic separation of xanthine-5' monophosphate (XMP). This assay measures the conversion of inosine 5'-monophosphate (IMP) to XMP in the presence of cofactor (nicotinamide adenine dinucleotide) followed by quantification of XMP by HPLC-UV and was revalidated in our laboratory.¹⁸

Peripheral blood mononuclear cells were isolated from whole blood using CPT Cell Preparation Tubes (Becton Dickinson, Franklin Lakes, NJ). The cell fraction was washed once with cold phosphate-buffered saline (pH 7.4) and suspended in 1.0 mL of the buffer. The cells were then counted and assessed for viability using a hemocytometer and light microscopy, pelleted by centrifugation and then lysed, with deionized water, at a density of 5×10^6 cells/mL, stored at -20°C , and analyzed within 4 weeks of blood collection. After two freeze–thaw cycles, the lysed cell suspension was centrifuged at 10,000 g and the supernatant (50 μL) was incubated with 1000 $\mu\text{mol/L}$ IMP, 1000 $\mu\text{mol/L}$ nicotinamide adenine dinucleotide, 0.04 mol/L sodium hydrogen phosphate, and 0.1 mol/L potassium chloride (pH 7.4) at 37°C for 2.5 hours, after which the reaction was stopped by adding 20 μL of 4 molar ice-cold perchloric acid. The deproteinized incubation mixture was then centrifuged at 10,000 g for 5 minutes. The supernatant (170 μL) was neutralized with 10 μL of 5 mol/L potassium carbonate, centrifuged at 10,000 g for 5 minutes, and 10 μL was injected onto the high-performance liquid chromatography column.

The chromatographic separation was performed on a 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 ultraviolet detector set at 254 nm. Chromatographic separation of individual analytes was achieved using a Hypersil ODS-2 (150 $\times$ 4.6 mm, 5 μm particle size; Thermo Electron Corp., San Jose, CA) maintained at 30°C . Mobile phase consisted of an isocratic composition of 3:97% v/v methanol:50 mM

potassium dihydrogen phosphate and 7 mM tetra-n-butyl ammonium hydrogen phosphate (pH 4.5) maintained at a flow rate of 0.6 mL/min for 20 minutes.

Calibrator concentrations ranged from 1 to 750 nmol/mL XMP prepared in the incubation buffer. The calibration curves were linear and the coefficient of determination (r^2) ranged from 0.9997 to 0.9999. Accuracy and precision of the assay was determined by measuring the XMP concentration in quality control standards at 5, 50, and 500 nmol/mL XMP. The accuracy of the assay ranged from 93.0 to 112.6% (mean $\pm$ standard deviation, 105.5% $\pm$ 6.2%) and coefficient of variation of the measurements was less than 5%.

The protein content of the cell lysate was determined using a bicinchoninic acid protein assay kit (Pierce, Rockford, IL). The rate of reaction for IMPDH activity was calculated by dividing the number of micromoles of XMP formed by the time of incubation and the protein content in the same volume of cell lysate (50 μ L) that was used for the incubation experiment (expressed as a unit of nmol XMP/h/ μ g_{protein}).

Western Blot for Characterization of Inosine Monophosphate Dehydrogenase II

Western blotting was used to determine the IMPDH-II content of cell lysates in selected predose patient samples having lowest IMPDH activity for patients with diabetes and highest IMPDH activity for nondiabetic patients. Cell lysates were prepared according to the method described in the determination of IMPDH activity. The lysates were subjected to centrifugation at 12,000 g for 15 minutes at 4°C to remove insoluble precipitates, were resolved (15 μ g of total protein each) by 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis in a minigel apparatus, and transferred electrophoretically to nitrocellulose membranes (Bio-Rad, Hercules, CA). As positive control, 0.1 μ g recombinant IMPDH-II (Sigma-Aldrich, St. Louis, MO) was analyzed simultaneously. Also, monoclonal antibody to glyceraldehyde-3-phosphate dehydrogenase protein (Abcam, Cambridge, MA) was used as a loading control. After nonspecific binding sites were blocked with 5% nonfat milk, the blots were incubated with a monoclonal antibody against IMPDH-II (Antibody Solutions, Palo Alto, CA). The primary antibody was subsequently localized with goat anti-mouse IgG conjugated with horseradish peroxidase.¹⁹ Horseradish peroxidase activity was detected with a chemiluminescent kit (SuperSignal West Pico, Pierce Biotechnology, Inc., Rockford, IL). The chemiluminescent signal was captured by Kodak Image Station 2000–(Eastman Kodak Company, Rochester, NY).

Data Analysis

WinNonlin software version 5.0.1 (Pharsight, Mountain View, CA) was used to perform the noncompartmental analysis (model 200) to obtain basic pharmacokinetic parameters, including area under the concentration–time curve from 0 to 12 hours (AUC_{0-12}) by the linear trapezoidal method, minimum plasma concentration, maximum plasma concentration, and time to reach maximum plasma concentration (T_{max}) for all four analytes. Mycophenolic acid unbound fraction was calculated as unbound MPA concentration divided by total MPA concentration and expressed as a percentage. Where dose-normalized values are reported, the plasma concentration or pharmacokinetic parameter was normalized to a dose of 720 mg EC-MPS twice a day. No attempt was made to report elimination half-life

because elimination rate constant could not be accurately determined as a result of the presence of enterohepatic recirculation in the terminal phase of the concentration–time curve.

All statistical tests were performed using SPSS software version 11.5 (SPSS, Chicago, IL) and a P value <0.05 was considered significant. For all parameters, a Kolmogorov-Smirnov test with Lilliefors's correction was performed to determine normality of data distribution. All nonnormally distributed data are reported as median and analyzed by the Mann-Whitney U test or were transformed to natural logarithm values, reported as geometric mean, and analyzed by independent-samples t test. Backtransformed values of standard deviation are reported for all nonnormally distributed data. The differences in proportions were analyzed by χ^2 test.

RESULTS

Nine diabetic (D) and nine nondiabetic (ND) patients completed the study. Five of the diabetic patients had type 1 and four had type 2 diabetes. All patients with diabetes had required transplantation as a result of kidney failure caused by diabetic nephropathy and had thus been diabetic for many years (exact duration was not known). Four of the nine patients with diabetes were considered to have uncontrolled diabetes based on their hemoglobin A_{1c} levels and history of fluctuations in blood glucose levels. There were no differences in age, weight, ethnic origin, or estimated creatinine clearance between the two groups, but serum glucose and hemoglobin A_{1c} was significantly higher in the patients with diabetes (Table 1).

The mean $\pm$ standard deviation of EC-MPS dose was 960 ± 360 mg/day in diabetic and 1120 ± 379 mg/day in the nondiabetic groups ($P = 0.37$). The difference in the average dose was because of different EC-MPS doses in two of nine patient pairs. Cyclosporine average daily dose (range) was 112 (25–250) mg in patients with diabetes and 56 (50–75) mg in nondiabetics. Two of four patients with diabetes on cyclosporine were also receiving ketoconazole in comparison with four of four nondiabetics resulting in a higher cyclosporine dose in patients with diabetes. Daily dose of tacrolimus was 5.6 (4–8) mg in patients with diabetes and 6.4 (4–14) mg in nondiabetics. Furthermore, daily dose of oral prednisone was 5.6 (3–10) mg in patients with diabetes and 5.6 (5–10) mg in nondiabetics.

In addition to immunosuppressive agents, patients with diabetes were receiving an average of 7.7 other medications as compared with 6.2 for the nondiabetic group. The most frequently used group of medications were angiotensin-converting enzyme inhibitors (four D, three ND), azole antifungal agents (three D, four ND), beta-blockers (six D, seven ND), calcium channel blocker (five D, four ND), H₂ antagonist (two D, three ND), loop diuretic furosemide (three D, two ND), proton pump inhibitors (five D, three ND), statins (six D, six ND), and sulfamethoxazole/trimethoprim (five D, five ND). The only medication class taken exclusively by patients with diabetes was insulin (short, intermediate, or long-acting).

Gastric Emptying and Glomerular Filtration Rate

Concentration–time profiles revealed that three patients with diabetes and one nondiabetic patient had noticeable delays in acetaminophen absorption. The mean time to reach

maximum concentration of acetaminophen was approximately 20 minutes longer in the diabetic group (59.2 ± 46.9 minutes versus 39.9 ± 35.1 minutes in the nondiabetic patients; $P = 0.22$). There was a trend to lower acetaminophen concentration in the diabetic group at 30 minutes postdose (8.7 ± 5.8 versus 13.9 ± 5.7 mg/L in the nondiabetic group, $P = 0.07$), as a result of delay in gastric emptying, but acetaminophen AUC did not differ between groups.

Glomerular filtration rate, as measured by iothexol clearance, was numerically lower in the patients with diabetes (56.0 ± 17.1 versus 68.3 ± 25.9 mL/min/1.73 m² in the nondiabetic group, $P = 0.25$).

Pharmacokinetics of Mycophenolic Acid and Mycophenolic Acid Metabolites

All pharmacokinetic studies were performed after the morning dose of EC-MPS and morning dose-normalized parameters are reported for total and unbound MPA (Table 2) or MPA metabolites (Table 3). In addition, the average dose-normalized plasma concentration–time profiles of total and unbound MPA, MPAG, and AcMPAG are shown in Figure 1. There were no significant differences in any pharmacokinetic parameter for total and unbound MPA between the diabetic and nondiabetic groups, indicating that the rate ($T_{\max}$) and extent (AUC_{0-12}) of exposure to total MPA and unbound MPA were comparable. Furthermore, the median (range) of MPA AUC_{0-12} before dose normalization was 36.7 (16.4–116.4) mg* h /L in patients with diabetes and 48.2 (34.9–80.1) mg* h /L in nondiabetic patients ($P = 0.49$). The only measurable difference observed was the ratio of MPAG to AcMPAG (based on all time points), which was significantly higher in patients with diabetes (Table 3).

Inosine Monophosphate Dehydrogenase Activity and Inosine Monophosphate Dehydrogenase II Protein Expression

IMPDH activity was significantly lower in patients with diabetes versus the nondiabetic group throughout the 12-hour dosing interval ($P < 0.0001$) (Fig. 2A). Mean $\pm$ standard deviation of IMPDH activity was 17.5 ± 2.8 nmol XMP/h/ μ g_{protein} in patients with diabetes versus 46.6 ± 2.5 nmol XMP/h/ μ g_{protein} in nondiabetics. A comparison of IMPDH activity at predose baseline (corresponding to morning trough concentration of MPA) revealed that IMPDH activity was significantly different between the patients with diabetes and nondiabetic patients (14.4 ± 1.9 versus 53.6 ± 2.1 XMP/h/ μ g_{protein}; $P < 0.01$). The highest degree of IMPDH inhibition (lowest IMPDH activity) was observed at 2 hours after ECMPS dose (9.1 ± 2.5 versus 29.9 ± 2.2 XMP/h/ μ g_{protein} in D and ND, respectively, $P = 0.012$) and the lowest IMPDH inhibition (highest IMPDH activity) was observed at 12 hours postdose (20.1 ± 3.3 versus 53.7 ± 3.5 XMP/h/ μ g_{protein} in D and ND, respectively, $P = 0.127$).

The IMPDH activity was compared between patients with diabetes and nondiabetic patients receiving concomitant cyclosporine or tacrolimus therapy (Fig. 2B). The IMPDH activity was pronouncedly lower in the patients with diabetes on cyclosporine ($P < 0.0001$) than on tacrolimus ($P < 0.05$). Pharmacokinetics parameters, MPA, fMPA, or AcMPAG concentrations were comparable between patients with diabetes and nondiabetic patients

coadministered either cyclosporine or tacrolimus; however, the concentration of MPAG was significantly higher (26%, $P < 0.002$) in patients with diabetes on tacrolimus.

Figure 3 illustrates the Western blot analysis for the IMPDH-II protein content of selected samples collected at predose baseline (0 hours) in four patients with diabetes, four nondiabetic patients, two apparently healthy control subjects, and the positive control (recombinant human IMPDH-II protein). Of the four nondiabetic patients, three had higher expression levels for IMPDH-II than the diabetic recipients (mean $\pm$ standard deviation of relative intensity was 10286 ± 1133 in patients with diabetes, 26166 ± 9371 in nondiabetics, and 19068 ± 5656 for positive control). The relative intensity of IMPDH-II protein in two apparently healthy volunteers is not conclusive and requires further investigation in a larger number patients, healthy subjects and nontransplanted patients with diabetes.

DISCUSSION

These results indicate that the rate and extent of MPA absorption using the EC-MPS formulation in stable kidney transplant patients is not affected by the presence or absence of diabetes. IMPDH activity, however, was found to be significantly lower in patients with diabetes compared with nondiabetics.

The finding that the rate of absorption of MPA from EC-MPS, as judged by $T_{\max}$, was similar in patients with diabetes and nondiabetic patients is in contrast to previous observations in patients receiving MMF.^{6,7,20,21} One possible reason for this discrepancy is that the absorption of MPA from the enteric-coated formulation begins in the small intestine instead of the stomach, and thus delayed gastric emptying (as indicated in our population by some delay in acetaminophen absorption) may have little or no effect on EC-MPS absorption rate. The number of the subjects included in this study is rather small; however, this sample size is usual for a 12-hour pharmacokinetic study. The main pharmacokinetic parameters of interest (total MPA AUC_{0-12} , maximum plasma concentration, and $T_{\max}$) were very similar between the two groups (P value approximately 0.75) and a post hoc sample size analysis showed more than 600 patients in each group will be needed to achieve statistical significance at 80% power.

Mycophenolic acid undergoes biotransformation by phase II conjugation to glucuronide metabolites, AcMPAG and MPAG, which are eliminated both renally and hepatically (MPAG undergoes enterohepatic recirculation). Although altered hepatic clearance and nephropathy have been reported in diabetics,²² we observed no differences in AcMPAG and MPAG AUC_{0-12} between the two groups, indicating that diabetes did not alter renal or biliary elimination. Similar to our previous study using MMF,⁶ the ratio of MPAG to AcMPAG was significantly higher in patients with diabetes, a finding that might relate to lower kidney function (glomerular filtration rate values on average were 11 mL/min lower) in these patients. Also, drug metabolism enzymes and transporters can be affected by diabetes^{23,24} therefore, to further address this difference, one must study the effect of diabetes mellitus on the glucuronidation enzymes, UGT1A8/9 and UGT2B7, responsible for the conversion MPA to MPAG and AcMPAG, respectively, and multidrug resistance protein 2 that effluxes MPAG from the liver into the gastrointestinal tract.

It is well known that diabetes alters cell-mediated immunity, lowers production of some cytokines, and suppresses T and B lymphocyte function.^{25–29} In the context of transplantation, diabetic transplant recipients are more prone to opportunistic infections. Recently, Lansang et al¹ showed that the risk of developing bacterial or viral infections requiring hospitalization was 43% higher in patients who were diabetic before transplantation than those who were nondiabetic and was 77% higher in patients who developed posttransplant diabetes mellitus. Another study showed that the odds ratio for developing an opportunistic fungal infection requiring hospitalization was 2.4 greater in patients for whom diabetes was the cause of end-stage renal disease versus other etiologies.¹¹

Both B and T lymphocytes are critically dependent on the de novo guanosine nucleotide synthesis pathway catalyzed by IMPDH for replication. In our population, IMPDH activity was significantly lower in patients with diabetes versus nondiabetic patients although the mean daily dose of EC-MPS was slightly higher in nondiabetics and there was no difference in the exposure to total or unbound MPA. We also have data from an ongoing study suggesting that another pharmacodynamic marker of MPA activity, expression of phenotypic marker CD95+ on B-lymphocytes (CD19+),³⁰ is significantly reduced in diabetic kidney transplant recipients (unpublished observation), which is consistent with the observation on IMPDH activity.

Additionally, in patients with diabetes, the trough concentration of concomitant calcineurin inhibitor therapy did not differ between the two groups. Glomerular filtration rate measured by the use of iohexol clearance on average was 11 mL/min lower in patients with diabetes, possibly because of detrimental effects of diabetes on the kidney function, but it is unlikely that lower kidney function can generate such significant effect in IMPDH activity. Also, it is unclear why the IMPDH activity was so much lower in patients with diabetes on cyclosporine than in patients on tacrolimus.

The only medication taken exclusively by patients with diabetes was short, intermediate, or long-acting insulin. Very little is known about the effect of insulin on MPA pharmacokinetics or pharmacodynamics; however, it appears that there is a relationship between insulin and IMPDH regulation. Whitehead et al³¹ have identified IMPDH as a new molecular target in the insulin signal transduction pathway and found that in vitro, insulin stimulates phosphorylation of IMPDH I or II and the translocation of IMPDH to lipid bodies. It is therefore important to compare the IMPDH mRNA expression and activity in diabetic transplant recipients on insulin and those on oral hypoglycemic agents to determine whether the lower IMPDH activity observed in this study is related to diabetic condition or insulin use.

In this study, IMPDH activity was measured in peripheral blood mononuclear cells using a modified version of an assay reported by Glander et al.¹⁷ Several different assay methodologies have been reported for the measurement of IMPDH activity in different cell types (ie, red blood, mononuclear or CD3+ cells)^{17,31–35}; however, because of differences in the assay conditions, it is difficult to compare the values of IMPDH activity between publications. Initially, we have tried to follow the assay published by Glander et al¹⁷;

however, we had to implement some modifications. Nevertheless, the effect of substrate, cofactor, and enzyme concentration was studied on the rate of XMP production and the final assay condition, with the exception of chromatographic separation and type of cell preparation tube, was similar to the original assay.¹⁸ The values of IMPDH activity observed in our study are expressed as nmol XMP/h/ $\mu\text{g}_{\text{protein}}$ or the activity in 50 mL of the cell lysate normalized for the total protein concentration in the same volume. In contrast, the IMPDH activity levels reported by Glander et al¹⁷ are expressed as nmol XMP/h/ $\text{mg}_{\text{protein}}$; the IMPDH activity in 50 μL of cell lysate normalized to the protein concentration in 1 mL cell lysate (personal communication with Dr. Glander), hence the difference in the unit of measurements.

The Western blot data in selected patients indicates that cellular expression of IMPDH-II was higher in nondiabetics as compared with patients with diabetes. However, it must be noted that MPA inhibits both IMPDH-I and -II isoforms,³⁶ and the IMPDH activity assay reported in this article also measures the activity of both IMPDH-I and -II. Although the data on IMPDH II protein expression in patients with diabetes are preliminary, it may suggest that diabetes not only affects IMPDH activity, but may to some extent also alter the expression of key enzymes that define this aspect of immune response. Western blot analysis is a semiquantifiable technique; therefore, this observation requires further validation by measuring both IMPDH type I and II protein levels by enzyme-linked immunosorbent or comparable assays in a larger number of transplant recipients, nontransplanted diabetic subjects with renal insufficiency, and healthy control subjects before any definite conclusion can be made on the effect of diabetes on the expression of IMPDH protein.

In conclusion, the pharmacokinetic parameters of MPA are minimally affected by diabetes when administered either a MMF or EC-MPS formulation. However, this study is the first to demonstrate that IMPDH catalytic activity is lower in diabetic than nondiabetic kidney transplant recipients. It would be of interest to study the mechanism of reduced IMPDH activity by diabetes. This observation and its association with other biomarkers of cellular or humoral immunity necessitate further validation in the form of a longitudinal study including de novo diabetic and nondiabetic kidney transplant recipients.

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FIGURE 1.

Average concentration-time profile for (A) total mycophenolic acid, (B) unbound mycophenolic acid, (C) mycophenolic acid-glucuronide, and (D) acyl-mycophenolic acid-glucuronide in diabetic (n = 9) and nondiabetic (n = 9) kidney transplant recipients. Concentrations are normalized to a dose of 720 mg enteric-coated formulation of the sodium salt derivative of mycophenolic acid twice a day (all data are geometric mean and error bars show standard deviation of the mean).

FIGURE 2.

A, Inosine 5'-monophosphate dehydrogenase (IMPDH) catalytic activity versus time postdose in diabetic (n = 9) and nondiabetic (n = 9) kidney transplant recipients. B, IMPDH catalytic activity in patients with diabetes and nondiabetic patients on concomitant therapy with cyclosporine (four diabetic, four nondiabetics) or tacrolimus (five diabetic, five nondiabetic) (all data are geometric mean and error bars show standard deviation of the mean).

FIGURE 3.

Western blot of inosine 5'-monophosphate dehydrogenase (IMPDH) II protein expression in patients with diabetes ($n = 4$) and nondiabetic ($n = 4$) patients, healthy volunteers ($n = 2$), and positive control (recombinant human IMPDH-II protein) using anti-IMPDH-II antibody.

TABLE 1

Patient Demographics and Baseline Characteristics

	Diabetic (n = 9)	Nondiabetic (n = 9)
Age (years)	46.6 ± 14.4	43.9 ± 12.4
Weight (kg)	89.8 ± 18.5	86.9 ± 15.7
Time posttransplant (days)	1119 ± 833	1282 ± 868
Ethnicity		
White	6	4
Hispanic	1	2
Black	2	3
Type 1/type 2 diabetes	5/4	N/A
Controlled/uncontrolled diabetes	5/4	N/A
Creatinine clearance* (mL/min)	70.9 ± 28.6	77.5 ± 22.4
Iohexol clearance (mL/min/1.73 m ²)	56.0 ± 17.1	68.3 ± 25.9
Serum glucose (g/L)	169 ± 103	93 ± 18 [†]
Hemoglobin A _{1c} (%)	8.3 ± 1.8	5.4 ± 9.6 [†]
EC-MPS daily dose (mg)	960 ± 360	1120 ± 379
Cyclosporine/tacrolimus	4/5	4/5
Median cyclosporine trough concentration (μg/L) [‡]	46.1 (22.5, 76.7)	72.3 (36.8, 200.5)
Median tacrolimus trough concentration (μg/L) [‡]	5.8 (3.9, 8.9)	4.9 (3.8, 9.8)

All continuous variables expressed as mean ± standard deviation unless otherwise stated; data in parentheses indicate minimum and maximum values.

EC-MPS, enteric-coated formulation of the sodium salt derivative of mycophenolic acid; N/A, not applicable.

* Estimated using Cockcroft and Gault equation.

[†] $P < 0.05$.

[‡] Cyclosporine and tacrolimus concentrations were measured using liquid chromatography–tandem mass spectrometry.

TABLE 2

Pharmacokinetic Parameters for Total and Unbound MPA Normalized to a Dose of 720 mg EC-MPS Twice a Day

	Diabetic (n = 9)	Nondiabetic (n = 9)	P Value
Total MPA			
C ₀ (mg/L)	3.3 (1.3, 7.0)	2.3 (1.3, 5.9)	0.30
C ₁₂ (mg/L)	3.2 (2.0, 4.9)	2.6 (1.5, 4.8)	0.47
C _{max} (mg/L)	18.1 (5.7, 38.7)	22.2 (5.5, 45.5)	0.74
T _{max} (h)	1.8 (1.5, 7.1)	2.1 (1.0, 7.0)	0.91
AUC ₀₋₁₂ (mg* ^h /L)	73.5 (32.9, 116.4)	69.8 (48.3, 92.5)	0.75
Unbound MPA			
C ₀ (μg/L)	21.0 (4.3, 50.5)	24.4 (5.6, 77.5)	0.70
C ₁₂ (μg/L)	25.1 (13.2, 36.9)	11.2 (5.9, 43.9)	0.27
C _{max} (μg/L)	237.0 (79.2, 654.0)	180.0 (74.4, 387.0)	0.70
T _{max} (hours)	3.0 (1.5, 7.1)	2.0 (0.7, 7.0)	0.91
AUC ₀₋₁₂ (mg* ^h /L)	548 (142, 852)	466 (232, 852)	0.53
F _U (%)	0.84 (0.31, 1.36)	0.74 (0.30, 1.77)	0.76

All data expressed as median (minimum, maximum).

MPA, mycophenolic acid; EC-MPS, enteric-coated formulation of the sodium salt derivative of mycophenolic acid; C₀, morning trough plasma concentration; C₁₂, evening trough plasma concentration; C_{max}, maximum plasma concentration; T_{max}, time to maximum plasma concentration; AUC₀₋₁₂, area under the concentration–time curve from 0 to 12 hours; F_U (%), mycophenolic acid fraction unbound expressed as percentage.

TABLE 3

Pharmacokinetic Parameters for MPAG and AcMPAG Normalized to a Dose of 720 mg EC-MPS Twice a Day

	Diabetic (n = 9)	Nondiabetic (n = 9)	<i>P</i> Value
MPAG			
C ₀ (mg/L)	71.6 (39.1, 177.1)	42.2 (29.0, 131.6)	0.18
AUC ₀₋₁₂ (mg [*] h/L)	982.2 (529.2, 1901.4)	669.9 (320.1, 1776.7)	0.38
AcMPAG			
C ₀ (mg/L)	2.6 (1.4, 5.1)	1.9 (0.7, 5.8)	0.82
AUC ₀₋₁₂ (mg [*] h/L)	35.5 (19.6, 76.7)	28.3 (10.6, 82.8)	0.98
Concentration ratio			
MPAG:AcMPAG	25.5 (12.0, 70.3)	23.5 (12.7, 53.2)	<0.01

All data expressed as median (minimum, maximum).

MPAG, mycophenolic acid-glucuronide; AcMPAG, acyl-mycophenolic acid-glucuronide; EC-MPS, enteric-coated formulation of the sodium salt derivative of mycophenolic acid; C₀, morning trough plasma concentration; AUC₀₋₁₂, area under the concentration–time curve from 0 to 12 hours.