Intraindividual and Interindividual Variations in the Pharmacokinetics of Mycophenolic Acid in Liver Transplant Patients

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The authors evaluated the intraindividual and interindividual variations in the pharmacokinetics of mycophenolic acid after oral administration of mycophenolate mofetil in 10 liver transplant patients. Mycophenolic acid and its metabolite, mycophenolic acid glucuronide, were measured in plasma and urine by high-pressure liquid chromatography. The plasma protein binding of mycophenolic acid was determined by ultrafiltration. The maximum concentration of mycophenolic acid in plasma increased significantly (P ≤ .05) with time from 9.1 ± 7.2 μg/mL (<1 week) to 36.7 ± 15.6 μg/mL (1 month). The area under the plasma concentration versus time curve of mycophenolic acid also increased significantly with time, from 50.8 ± 42.1 μg•h/mL to 118.0 ± 57.6 μg•h/mL (P ≤ .05). The plasma protein binding of mycophenolic acid increased from 92% to 98%, and the apparent oral clearance [CL/F] decreased from 32.9 ± 21.4 L/h during the first study period to 9.0 ± 4.4 L/h (P ≤ .05) during the third study period. The apparent intrinsic clearance of mycophenolic acid did not change significantly over time. The ratio of the area under the curve of mycophenolic acid glucuronide to mycophenolic acid in plasma decreased with time (25.5 ± 21.2 vs 8.0 ± 3.3) but did not reach statistical significance. The increased binding of mycophenolic acid to plasma proteins with time after transplantation appeared to contribute to the intraindividual variation, whereas differences in the ability of the liver to metabolize mycophenolic acid between patients appear to contribute to the large interindividual variation in the pharmacokinetics of mycophenolic acid. The observations in this study support the concept of measuring the unbound concentration of mycophenolic acid to optimize immunosuppressive drug therapy with mycophenolic acid.

Keywords: *Mycophenolic acid; mycophenolate mofetil; immunosuppressive drugs; pharmacokinetics; mycophenolic acid glucuronide; interindividual variations; intraindividual variations*

Mycophenolate mofetil (MMF, Cellcept), a morpholinoethyl ester prodrug of mycophenolic acid (MPA), is an immunosuppressive drug that is approved for the prophylaxis of acute rejection in renal transplant patients. Currently, MMF is used in combination with tacrolimus and cyclosporine. Mycophenolic acid is a reversible, noncompetitive inhibitor of ionosine monophosphate dehydrogenase (IMPDH), a key enzyme in the de novo purine biosynthesis of proliferating T and B lymphocytes.[1-3] Mycophenolic acid is converted to a glucuronide conjugate, mycophenolic acid glucuronide (MPAG), that is excreted in the bile and urine. Large variability in the pharmacokinetics of MPA has been reported in transplant patients.[4]

Large interindividual and intraindividual variability in the pharmacokinetics of several drugs has been reported in organ transplant patients.[5-8] This variability may be due to changes in absorption, distribution, me-

tabolism, and elimination of drugs in this patient population. Factors that contribute to these changes include (1) changes in gut and biliary function; (2) changes in the plasma protein binding; (3) changes in the functional capacity of the liver to metabolize drugs; (4) changes in the circulating levels of endogenous mediators such as endotoxin, cytokines, and nitric oxide, which could affect drug metabolism; (5) changes in the biliary transport of compounds; and (6) changes in renal function due to the concurrent use of nephrotoxic drugs such as tacrolimus and cyclosporine.

MPA is extensively bound (97%-98%) to human serum albumin.[9] Studies in renal transplant patients with renal dysfunction have also shown an increase in the unbound fraction of MPA due to changes in albumin concentrations.[10,11] Albumin concentrations are normally low in patients with liver disease. Subsequent to a liver transplantation (LTx), albumin concentrations increase toward normal values as the new transplanted liver synthesizes albumin.[5,12,13] Changes in albumin concentrations and a simultaneous reduction in plasma bilirubin concentration are expected to increase the plasma protein binding of mycophenolic acid in liver transplant patients. Based on the pharmacokinetics of MPA in renal transplant patients and the physiological changes observed in liver transplant patients, we hypothesized that the plasma protein binding of MPA will be low in LTx during the early posttransplant period but will increase with time after transplantation. We also hypothesized that there will be differences in the rate of conjugation of MPA within and between LTx patients, leading to altered clearance of MPA. In the present study, we evaluated the time-dependent changes in the pharmacokinetics of MPA in LTx patients during 3 different time periods.

METHODS

The protocol for this study was approved by the Institutional Review Board for Biomedical Research. Patients were recruited by primary care physicians. Prior to the study, the study protocol was explained to the patients, and informed consent was obtained.

Study Subjects

Male and female patients, between the ages of 31 and 60 years, who were receiving MMF as part of their postoperative immunosuppressive therapy participated in this study. All the patients were on tacrolimus and steroid therapy. Concomitant use of other medications generally prescribed to these patients included antac-

ids, antibacterial agents, and antiviral agents. Patients were excluded if they were pregnant or were receiving drugs known to inhibit or induce (other than steroids) hepatic drug-metabolizing enzymes during the study period. Food and water intake was not restricted during the study periods.

Study Design

The pharmacokinetics of MPA was evaluated on 3 separate occasions ($\leq$1 week, >1 week and $\leq$2 weeks, and $\geq$3 weeks and $\leq$6 weeks) after LTx. The patients were studied while in the intensive care unit (ICU) within the hospital or at the outpatient clinic. Blood (2.5 mL) samples were collected in Vacutainers with EDTA as anticoagulant immediately before and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours after the regular oral dose of MMF (0.5-1 g bid). Blood samples were centrifuged at room temperature, and the plasma was separated and frozen at –70°C and analyzed for MPA and MPAG by high-pressure liquid chromatography (HPLC). Urine was collected for the entire 12-hour study period. Urine was aliquotted and frozen at –70°C until analysis of MPA and MPAG by HPLC. Biochemical parameters indicative of liver and renal function (aspartate aminotransferase [AST], alanine aminotransferase [ALT], bilirubin, and albumin; blood urea nitrogen and serum creatinine) were measured as part of postoperative care in these patients.

Laboratory Analysis

The HPLC assay procedures for measuring MPA and MPAG were developed in our laboratory and reported earlier.[14] The concentration of MPA in plasma and urine was measured after a solid-phase extraction (Sep-pak 1-mL C18 cartridges, Waters Corp, Milford, Mass). To 250 µL of the sample, 50 µL of internal standard (diazepam, 15 ng/mL) and 1 mL of 0.1N HCl were added, and the sample was vortexed. The sample was then passed through a C18 Sep-pak cartridge (Waters Corp) previously conditioned with 2 mL methanol and 10 mL water. The components of interest were eluted with 2 mL methanol. The methanolic extract was dried under nitrogen. The dried sample was reconstituted in 125 µL of 60:40 water and acetonitrile. Then, 100 µL of this solution was injected onto the HPLC column. A HPLC column (LC-18, Pico-tag, Waters-5µ; 3.9 mm i.d.; 300 mm long) maintained at 50°C was used in an isocratic mode (1.2 mL/min) with a mobile phase of acidified H$_2$O (pH 4.5-5.0)/acetonitrile (59:41). Mycophenolic acid was monitored at 254 nm. The retention times for

MPA and the internal standard were 5.6 and 10.7 minutes, respectively. The standard curve was prepared in plasma at the following concentrations: 0, 0.25, 0.5, 1.0, 2.5, 5.0, 10.0, and 25.0 µg/mL. The coefficient of variation of this assay was 4.8% to 8.6% (0.25-25 µg/mL). For the measurement of MPAG in the plasma and urine, 20 µL of plasma or urine (diluted 20 times with deionized water), 50 µL of internal standard (0.15 µg/mL phenolphthalein glucuronide), and 50 µL of acetonitrile were mixed, vortexed, and centrifuged (13,000 rpm) for 3 minutes. To 25 µL of supernatant, 25 µL of a mixture of mobile phase was added, and 30 µL of this solution was injected onto the HPLC column. A Hypersil BDS C-18 column (5 µ; 4.6 mm i.d.; 250 mm long; Alltech Association Inc, Deerfield, Ill,) was used, with a mobile phase composition of acidified water (591 µL of 85% of orthophosphoric acid in 1 L of DI water)/acetonitrile (74:26) in an isocratic mode at a flow rate of 1.1 mL/min. Mycophenolic acid glucuronide and the phenolphthalein glucuronide were monitored at 254 nm. The retention times for MPAG and phenolphthalein glucuronide were 10.8 and 12.2 minutes, respectively. The standard curve for MPAG was prepared in plasma at the following concentrations: 0, 2.5, 5.0, 12.5, 25.0, 50, 100, and 200 µg/mL. Samples with concentration values that exceeded 200 µg/mL were diluted and reanalyzed. The coefficient of variation was 3.7% at 25 µg/mL and 2.7% at 100 µg/mL.

The plasma protein binding of MPA was determined by ultrafiltration using Amicon filters (1 mL capacity, 30,000 molecular weight cutoff; Millipore Corporation, Bedford, Mass). Four to 5 plasma samples representing approximately every other time point in the pharmacokinetic study were pooled, and MPA in methanol (<5%) was added to achieve an approximate total concentration of 25 to 30 µg/mL. The total plasma concentration of MPA was measured in 50 µL of this sample. The rest of the sample was subjected to ultrafiltration using a Millipore ultrafiltrate device with centrifugation of the sample at 3000 rpm for approximately 1 hour at room temperature. The concentration of MPA in the filtrate and in the plasma was measured by HPLC as described above. The unbound fraction of MPA was calculated by the following equation:

$$\text{Unbound fraction (fu)} = [(\text{unbound concentration of MPA in filtrate})/(\text{total concentration of MPA})].$$

The percent unbound was calculated as fu × 100. All the samples were analyzed in duplicate. Earlier studies have shown the unbound fraction of MPA to be constant up to at least 50 µg/mL of MPA.

Table I Biochemical Parameters in Liver Transplant Patients Over a Period of 1 Month After Transplantation

Parameter	First Study Period	Second Study Period	Third Study Period
Total bilirubin, mg/dL	0.8-10.8	0.4-10.2	0.4-1.6[a,b]
Albumin, g/dL	1.8-3.5	2.1-3.0	3.1-3.7[a,b]
Serum creatinine, mg/dL	0.5-1.3	0.6-1.5	0.7-1.5
ALT, IU/L	89-975	23-118[a]	18-88[a,b]
AST, IU/L	24-1016	13-40	12-48[a]

Biochemical parameters were measured in each of the liver transplant patients over a period of 1 month on 3 separate occasions: <1 week (first study), <2 weeks (second study), and <1 month (third study). Normal ranges: total bilirubin = 0.4-1.4 mg/dL, albumin = 3.5-4.0 g/dL, serum creatinine <1.2 mg/dL, alanine aminotransferase (ALT) <40 IU/L, and aspartate aminotransferase (AST) <40 IU/L. Values are expressed as ranges in the patients in each of the studies.
a. $P < .05$ vs first study.
b. $P < .05$ vs second study.

Pharmacokinetic and Statistical Analysis

Area under the plasma concentration-time curve (AUC_{0-12h}), apparent total oral clearance (CL/F), peak concentration (C_{max}), and time to peak concentration (t_{max}) of MPA in plasma were computed by standard noncompartmental methods with WinNonlin (Standard edition, version 1.5). The apparent intrinsic clearance (CL_{int}) was calculated as (CL/F)/fu because MPA is a low-clearance drug.[15,16]

The relationship between various pharmacokinetic parameters and biochemical indices was examined by simple linear regression analysis. Repeated-measures ANOVA (SAS, release 8.01) was used to compare the various calculated parameters between the 3 study periods. Results were considered to be statistically significant at $P \leq .05$.

RESULTS

Ten (6 male, 4 female) liver allograft recipients completed the entire study. The mean age of the subjects who participated in this study was 49 years. The mean body weight was 65.3 kg. Of the 10 subjects who completed the study, four had postnecrotic cirrhosis, 3 had primary sclerosing cholangitis, 2 had primary biliary cirrhosis, and 1 had hepatitis C. Hepatic biochemical parameters such as AST, ALT, and total bilirubin were above normal during the first study period and returned to the normal range in almost all the patients by
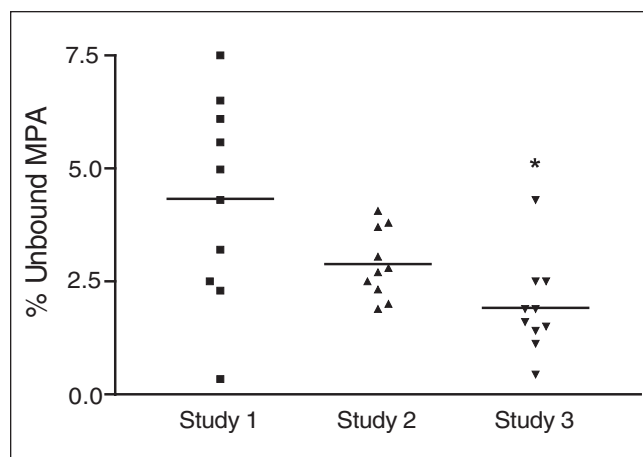
Figure 1. *Changes in percent unbound mycophenolic acid (MPA) in liver transplant patients over 1 month after transplantation. Percent unbound MPA was measured by ultrafiltration in liver transplant patients at <1 week (study period 1), <2 weeks (study period 2), and <1 month (study period 3) after liver transplantation. Each individual symbol represents the unbound MPA in each individual patient. Values expressed are individual values with a mean (horizontal bar). *P ≤ .05 versus study period 1.*
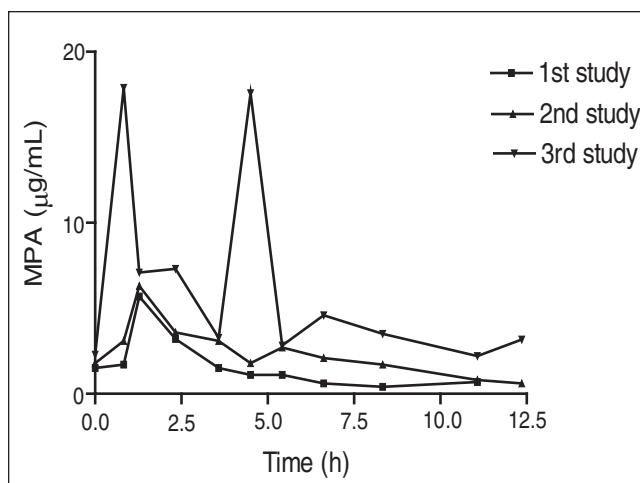

Figure 2. *Plasma concentration versus time profile of mycophenolic acid (MPA) in a liver transplant patient. Figure shows the plasma concentration versus time profile of MPA in the first, second, and third study periods in a liver transplant patient. A secondary peak, seen in the third kinetic study, is indicative of enterohepatic recycling (EHR).*
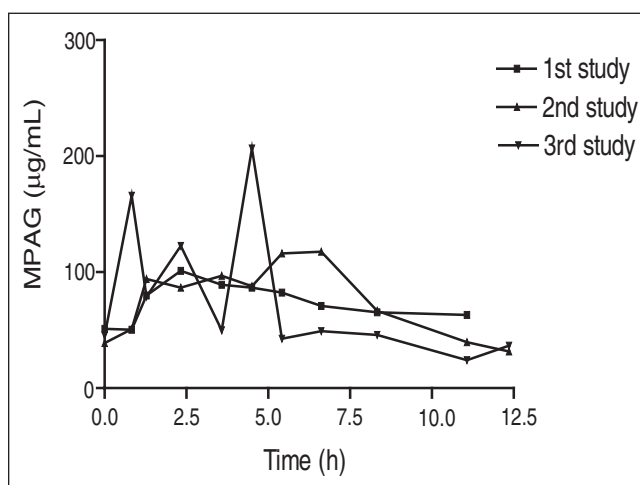
the third study period (Table I). Albumin levels were low (<3.5 mg/dL) in all the patients during the first study period and gradually increased with time, indicating normal synthetic function of the transplanted liver. Serum creatinine concentrations were relatively stable during the study period.

The percent unbound MPA ranged from 0.3% to 7.5% during the first study period. The mean percent unbound decreased from 4.3% during the first study period to 1.9% during the third study period (Figure 1, Table II), indicating increased binding of MPA to plasma proteins with time after transplantation.

Figures 2 and 3 show the plasma concentration versus time profile of MPA and MPAG in a patient after liver transplantation. A secondary peak in MPA was seen between 4 and 6 hours after MMF administration in 4 of 10 LTx patients during the third study period, indicative of possible enterohepatic circulation. The pharmacokinetic parameters of MPA and MPAG are summarized in Table II. The time to reach maximum concentration (t_{max}) did not change significantly (1.8 ± 1.2 vs 1.3 ± 0.7 h) between the first and the third study periods. The C_{max} increased approximately 3-fold (9.1 ± 7.2 vs 36.7 ± 15.6 µg/mL) with time. Similarly, the AUC increased significantly (approximately 2.5-fold) from 50.8 ± 42.1 to 118 ± 57.6 µg•h/mL. The apparent oral clearance of total MPA (CL/F) decreased from 32.9 ± 21.4 L/h during the first study period to 9.0 ± 4.4 L/h

Figure 3. *Plasma concentration versus time profile of mycophenolic acid glucuronide (MPAG) in a liver transplant patient. Figure shows the plasma concentration versus time profile of MPAG in the first, second, and third study periods in a liver transplant patient.*

during the third study period. The apparent CL_{int} of MPA varied among the patients during each study period, as indicated by a large standard deviation (see Figure 4). However, the CL_{int} of MPA was not significantly different at different study periods (Table II). The within-patient variability in the CL_{int} of MPA was less than 20%.

Table II Summary of Pharmacokinetic Parameters of Mycophenolic Acid (MPA) and Mycophenolic Acid Glucuronide (MPAG) in Liver Transplant Patients

Parameter	First Study Period	Second Study Period	Third Study Period
MPA			
C_{max}, μg/mL	9.1 ± 7.2	11.6 ± 6.7	36.7 ± 15.6[a]
t_{max}, h	1.8 ± 1.2	1.8 ± 1.4	1.3 ± 0.7
Percent unbound	4.3 ± 2.2	2.9 ± 0.8	1.9 ± 1.0[a]
AUC_{0-12}, μg•h/mL	50.8 ± 42.1	60.3 ± 38.5	118.0 ± 57.6[a]
CL/F, L/h	32.9 ± 21.4	25.9 ± 19.1	9.0 ± 4.4[a]
CL_{int}/F, L/h	920.2 ± 606.7	888.3 ± 549.5	769.8 ± 943.5
MPAG			
C_{max}, μg/mL	14.9 ± 97.5	113.1 ± 78.9	139.5 ± 130.9
t_{max}, h	4.7 ± 2.2	3.7 ± 1.9	3.4 ± 1.9
AUC_{0-12}, μg•h/mL	843.2 ± 630.3	737.4 ± 356.5	750.8 ± 419.6
MPAG AUC/ MPA AUC	25.5 ± 21.2	16.8 ± 13.2	8.0 ± 3.3
Percentage of dose as MPAG in urine	36.3 ± 10.9	31.1 ± 9.2	17.7 ± 10.3[a]

The values expressed are mean ± SD.

a. $P < .05$ vs first study period.

There was a significant correlation between the percent unbound MPA and the apparent oral clearance of the total drug. A significant correlation ($r = 0.88$) was observed between the unbound fraction and the plasma albumin concentrations, as determined by the following relationship:

$$1/fu = (1 + Ka \times fup \times Pt),$$

where Ka is the affinity constant, fup is the fraction of the total number of binding sites unoccupied, and Pt is the concentration of plasma albumin.

The relationship between the AUC_{0-12} of MPA and various biochemical indices was also examined. A moderate correlation was observed between the biochemical indices of bilirubin ($r^2 = 0.4$), albumin ($r^2 = 0.4$), ALT ($r^2 = 0.4$), AST ($r^2 = 0.5$), and MPA AUC_{0-12}. However, no correlation was observed between MPA AUC and serum creatinine concentrations.

The C_{max} and t_{max} for MPAG did not change over time. The AUC of MPAG was several folds higher than the AUC of MPA and did not change over time (Table II).
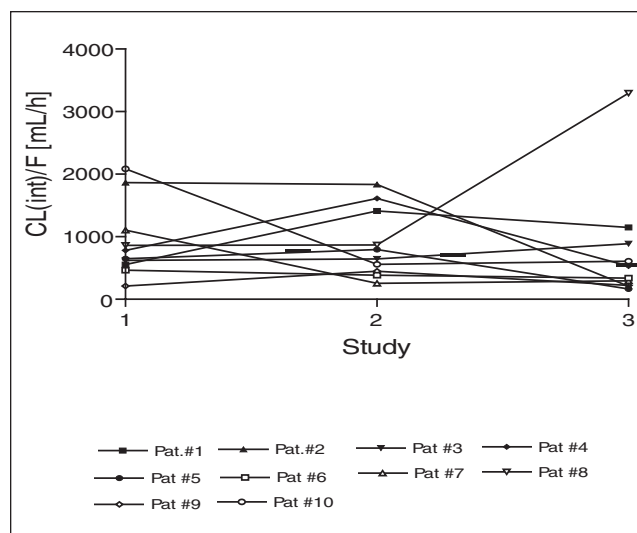

Figure 4.   Variations in the intrinsic clearance in liver transplant patients. Each symbol represents a subject. The apparent intrinsic clearance (CL_{int}) is calculated in each of the 3 study periods in liver transplant patients. Values expressed are individual values in each patient, with the horizontal bar representing the mean in each study.

The calculated ratio of the AUC of MPAG/MPA decreased from the first to the third study periods, but this did not achieve statistical significance. Poor correlations were observed between MPAG AUC or MPAG/MPA AUC ratio and serum creatinine. The percentage of the dose excreted in the urine over a dosing interval as MPAG was 36% during the first study period and 17% during the third study period.

DISCUSSION

Large interpatient variations in the pharmacokinetics of immunosuppressive drugs have been reported in transplant patients. Following transplantation, patients undergo marked changes in the physiological functions associated with the transplanted organs. Drug absorption, distribution, and elimination undergo a time-dependent transition from that associated with organ failure to that of the normal state. In addition, metabolism may be affected by endogenous mediators, such as cytokines and nitric oxide, and any hepatocellular damage caused by preservation/reperfusion. Biliary and renal dysfunction is common in transplant patients.[5,17,18]

Several reports have indicated interpatient variability with immunosuppressive agents such as tacrolimus and cyclosporine. The variability in the kinetics of cyclosporine has been related to factors such as changes in absorption, variability in liver or renal function,

age, food intake, and concurrent drug therapy.[5,19-21] Other studies have suggested that the wide interpatient variation in the doses needed to achieve an appropriate blood concentration of tacrolimus could be due to the variation in activity of drug-metabolizing enzymes (CYP3A) or drug transporter.[7,22,23]

The pharmacokinetics of MMF has been previously evaluated in renal and pancreas transplant patients. These studies have demonstrated wide variations in the kinetics of MPA when a fixed dose of MMF was administered to the patients.[15,16,24-31] However, limited information is available on the pharmacokinetics of MPA in liver transplant patients.[14,32-34]

In the present study, we evaluated the pharmacokinetics of MPA in LTx patients on 3 separate occasions in the immediate posttransplant period. In liver transplant patients, mycophenolate mofetil was rapidly absorbed after oral administration and readily converted to MPA. This is similar to what is observed in healthy volunteers and in kidney transplant patients.[15,16,25,26,28,29,35] MMF concentrations have been reported to be below detection limits in these patients within 1 hour after dosing. In our study, a 4-fold increase in the peak concentration (C_{max}) of MPA was observed between the first and the third study periods. This could be due to improved absorption of MPA over time. However, it was not possible to ascertain whether changes in absorption contributed to the changes observed in this study, as an intravenous formulation was not available at the time of the study.

MPA is highly bound to albumin in plasma.[9] In vitro studies have shown that the inhibition of IMPDH is dependent on the unbound concentration of MPA.[9] The concentration of albumin increases and the concentration of bilirubin decreases after liver transplantation. Our results indicate that plasma protein binding of MPA increased as the concentration of albumin increased and the concentration of bilirubin decreased with time in these subjects. Increased binding leads to decreased unbound fraction. This is typical of acidic drugs that show decreased binding in renal impairment, liver disease, and posttransplantation.[5,13,36] There was a 2.5-fold difference in the mean unbound fraction (fu) between the first study period and the third study period. This could have contributed to the observed differences in the pharmacokinetics of MPA between studies and within patients. The unbound fraction is similar to but more variable than what is reported in other transplant patient populations.[10,11,37]

The total AUC_{0-12} of MPA increased nearly 2.5-fold with time. The increase in total AUC correlated well with the decrease in percent unbound MPA. The total clearance (CL) of MPA decreased with increased bind-

ing, and this was significant between the first and the third study periods. The clearance of MPA similarly correlated with an increase in percent unbound MPA. Because MPA is a low-clearance drug, a decrease in the unbound fraction due to increased albumin, decreased bilirubin concentration, and other factors will decrease the clearance and increase total AUC. These results also agree with the isolated liver perfusion rat (IPRL) study, in which changes in the albumin concentrations produced drop changes in the clearance of MPA.[38] Time-dependent changes in the AUC of MPA have also been reported in kidney transplant patients.[30]

To identify other possible determinants of changes in MPA AUC, the relationships between biochemical indices such as ALT, AST, bilirubin, albumin, and MPA AUC were evaluated. AUC_{0-12} was moderately correlated to covariates such as ALT, AST, and bilirubin. No correlation was observed between MPA AUC_{0-12} and serum creatinine. However, the serum creatinine was fairly normal in our study population. We have previously observed a positive correlation between the MPAG/MPA AUC ratio and serum creatinine concentrations in patients with varying degrees of renal function. In a study evaluating the pharmacokinetics of MPA in adult kidney transplant recipients, AUC_{0-12} was positively predicted by both serum creatinine and serum albumin but not by time after transplantation, body weight, or trough concentrations.[28] In a pediatric liver transplant population, the trough MPA correlated well ($r^2 = 0.65$) with the AUC of MPA.[39] This is similar to our observation ($r^2 = 0.6$) in this study.

There was no change in the intrinsic clearance (CL_{int}) of MPA within the patients in this study. The lack of a significant change in the intrinsic clearance indicates that the inherent ability of the liver to metabolize and eliminate MPA did not change significantly over time in this study population. However, large variability was observed between patients. This large variability between patients could be due to differences in the amount of glucuronide-conjugating enzyme content in the liver or in the content of cosubstrate UDPGA in the liver or the effect of cytokines or nitric oxide on the metabolism of MPA. An understanding of the importance and contribution of these factors requires future studies.

It is known that MPA is predominantly converted to MPAG, a glucuronide conjugate.[15,16] Very high plasma concentrations of MPAG in comparison to MPA were observed in all the patients studied. There was a 2.5-fold difference in the ratio of the AUC of MPAG to MPA from the first study to the third study. The AUC of MPAG did not change with time, whereas the MPA AUC increased with time.

Higher trough plasma concentrations and AUCs of MPA have been reported in kidney transplant patients receiving tacrolimus in comparison to those on cyclosporine.[27] In our study, it was not possible to directly confirm this observation because all the patients were receiving tacrolimus. It was not possible to ascertain whether there were any changes in the enterohepatic recycling, which could have contributed to the changes seen in the kinetics of MPA over time.

In summary, we have observed time-dependent changes in the fu, AUC, and CL of MPA in liver transplant patients treated with MMF and tacrolimus. Our observations are consistent with published information in kidney transplant patients treated with cyclosporine and MMF. Our data also indicate large interpatient variability in the CL_{int} of MPA in liver transplant patients. The 2 factors that contributed to the overall variability in the pharmacokinetics of MPA are changes in plasma protein binding and changes in intrinsic clearance. Changes in plasma protein binding contributed to the intraindividual variability, whereas differences in plasma protein binding and differences in intrinsic clearance appear to have contributed to the interindividual variations. The large variability in the pharmacokinetics of MPA seen in different patients indicates the need for therapeutic monitoring of MPA in transplant patients. Because MPA is a low-clearance drug and is slightly bound to plasma proteins, free or unbound drug may be a better measure of drug exposure than the total drug. Future clinical studies that evaluate the unbound MPA concentration and the clinical outcomes are necessary.

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