Pharmacokinetics and Bioavailability of Mycophenolate Mofetil in Healthy Subjects after Single-Dose Oral and Intravenous Administration

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A randomized, crossover study of 12 healthy volunteers was conducted with single, 1.5-g doses of mycophenolate mofetil (MMF), a prodrug of mycophenolic acid (MPA), after oral and intravenous administration. During the intravenous infusion, phase systemic plasma clearance of MMF was ~10 L/min and the half-life ($t_{1/2}$) was a few minutes. After oral administration, however, plasma MMF was below quantitation limits at all times. The plasma MPA profile of oral MMF showed a sharp peak at ~1 hour and a secondary peak at 8 to 12 hours. Mean apparent plasma $t_{1/2}$ of MPA was similar for both routes (~17 hours). The area under the concentration–time curve (AUC) from time 0 to 24 hours was statistically higher for intravenous than for oral administration, but total AUC showed statistical equivalence (80–120 rule), with mean bioavailability of MPA from oral administration of MMF estimated as 94.1% relative to the intravenous route. Total plasma AUC of mycophenolic acid glucuronide (MPAG), the sole metabolite of MPA, was four- to five-fold higher than MPA. Total 48-hour MPAG recovery in urine was statistically equivalent for the two routes and represented a mean of 70% of administered drug; corresponding MPA recovery was less than 1%. Renal clearance (Cl_R) values required transport mechanisms for MPAG, but not for MPA. The Cl_R of MPAG was statistically higher after intravenous administration than oral administration. MMF administered orally undergoes rapid, complete absorption and essentially complete presystemic deesterification. There was presystemic removal of MPA, but enterohepatic circulation compensated for the first pass loss. Renal metabolism of MPA also may have occurred.

Mycophenolate mofetil (MMF; Syntex Research, Palo Alto, CA), an ester prodrug of the immunosuppressant mycophenolic acid (MPA), is a new molecular entity designed to increase the systemic bioavailability of MPA.[1] It is being developed for the prevention of rejection in patients receiving allogeneic transplants, with the initial focus on a formulation that can be administered orally to patients who have undergone renal transplantation[2] at a dosage of 1.0 to 1.5 g given twice daily.

An intravenous formulation also is being developed as an alternative dose form, and the availability of the intravenous formulation provided an opportunity to determine oral bioavailability of MMF in healthy subjects. Mycophenolic acid is the active immunosuppressant;[3] therefore, for purposes of clinical efficacy, MPA was the appropriate species to analyze for assessment of systemic bioavailability.

A crossover study of healthy volunteers was conducted to compare pharmacokinetics of MPA after both oral and intravenous administration of single 1.5-g doses of MMF. Mycophenolic acid is metabolized to a single glucuronidated metabolite, MPAG, which is then excreted in urine.[4] Measurements of MMF in plasma and of MPA and MPAG in both plasma and urine allowed the pharmacokinetics of MMF to be further characterized.

SUBJECTS AND METHODS

Participants

Twelve healthy, nonsmoking volunteers (six men and six women) between 20 and 45 years of age were

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recruited for the study. Participants weighed within 15% of the average weight for their sex, age, and height as determined by the Metropolitan Life Insurance Company Weight Tables; their medical histories were unremarkable, and results of physical examinations were normal.

At the beginning of the study, participants underwent hematologic, renal function (serum creatinine), and liver function tests. Results of these tests and of urinalyses were normal. Women were not admitted to the study unless the result of a serum pregnancy test taken within 3 days of administration of the first dose of MMF was negative; in addition, their sexual partners had to agree to use an effective means of contraception throughout the study and for 3 months after completion of the study.

Participants were excluded if they had a history of biliary tract disease, biliary tract surgery, or gastrointestinal surgery; if they recently had suffered from conditions that might alter gastrointestinal motility; or if they required any medication, including over-the-counter drugs or vitamins, from 1 week before the first dose of medication until the end of the study. Consumption of alcohol was prohibited from 72 hours before the first dose of MMF until the end of the study; consumption of caffeine was prohibited from 12 hours before each dose of study medication until 12 hours after each dose.

The study protocol was approved by an institutional review board (independent, FDA-constituted board), and all participants gave written informed consent before initiation of the study.

Study Protocol

The study was an open-label, two-period, single-dose, randomized crossover design, with a washout period of 7 to 10 days separating the dosing periods. During each of the treatment periods, participants fasted overnight for at least 10 hours with access to water only. Each participant then received 1.5 g of MMF given either orally as six 250-mg capsules with 225 mL of water or via the intravenous route as a solution of 6 mg/mL of MMF in 5% dextrose in water through a peripheral vein administered over a 60-minute period at a constant infusion rate of 4.2 mL per minute. Fasting continued until 4 hours after the start of drug administration, at which time a standard lunch was served. Standardized meals were given 8 and 10 hours after administration.

During the oral-dose phase, blood samples were drawn before and 20 and 40 minutes and 1, 1.25, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, and 48 hours after administration. During the intravenous-dose phase, samples were taken from the arm contralateral to that used for drug administration before the dose, and 20, 40, 60, 65, and 70 minutes and 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, and 48 hours after the start of the infusion. Samples collected for 90 minutes after oral administration and up to 30 minutes after intravenous infusion were assayed for MMF, MPA, and MPAG, and were processed appropriately[5] to prevent degradation of MMF. All other plasma samples were stored frozen at −20 °C until assayed for MPA and MPAG.

A baseline urine sample was collected from each subject before administration of the study drug. Urine collections then were made over the following intervals after oral administration or the start of intravenous infusion: 0 to 2, 2 to 4, 4 to 8, 8 to 12, 12 to 24, 24 to 36, and 36 to 48 hours. The total volume of urine collected over each interval was recorded, and then 10-mL aliquots were removed and stored frozen until subsequent analysis for MPA and MPAG.

Safety was monitored by routine clinical laboratory tests conducted before and 48 hours after administration of MMF, by recording reported adverse events, and by conducting physical examinations before and after the study. During the intravenous period, the infusion site was assessed for signs of irritation.

Sample Analysis

Plasma samples were analyzed by high-performance liquid chromatography (HPLC) with ultraviolet detection using reported methods for MMF[5] and MPA and MPAG.[6]

Concentrations of MPA and MPAG in urine were determined by a method that used a BenchMate workstation (Model BSF1; Zymark, Hopkinton, MA), a Rabbit binary HPLC system (Rainin Instruments, Woburn, MA), and a Spectraflow 783 ultraviolet detector set at 254 nm (ABI Analytical, Foster City, CA) as follows.

Urine samples were diluted serially and gravimetrically by the BenchMate workstation with acetonitrile water (10:90, v/v) by factors of 10 for MPA and 50 for MPAG. After addition of an internal standard solution (RS-60461-000, Syntex Research, Palo Alto, CA for MPA; phenolphthalein mono-β-glucuronic acid sodium salt, Sigma Chemical Co, St Louis, MO for MPAG) to an aliquot of the diluted urine, 20-mL portions of the sample were injected into a HPLC system equipped with an Applied Biosystems Newguard RP-2, 15- × 3.2-mm guard column (Rainin Instruments, Woburn, MA) and an Adsorbosphere HS C-18, 5-mm, 150- × 46-mm chromatography column (Alltech, Deerfield, IL).

All HPLC analyses of MPA in a run were performed first, whereupon the mobile phase was changed and MPAG analyzed. The mobile phase for MPA was 41% acetonitrile in 0.05% aqueous phos-

phoric acid, and the mobile phase for MPAG was 23% acetonitrile in 0.05% aqueous phosphoric acid. The calibration ranges for the method in terms of the undiluted urine were 2.50 to 100 μg/mL for MPA (free acid) and 50.0 to 5,000 μg/mL for MPAG (anhydrous disodium salt). For MPA, all within-run and between-run coefficients of variation for calibration standards and quality-control urine samples were less than 11%, except for a 22% between-run coefficient of variation for the lowest calibration standard.

Recovery of MPA (ratio of found to nominal concentration expressed as a percentage) ranged from 92% to 110%. For MPAG, all coefficients of variation were less than 10%, and recovery ranged from 86% to 103%. The MPA and MPAG were stable in urine stored at room temperature (21–24°C) for at least 8 hours, refrigerated (1–4°C) for at least 15 days, on-instrument (12–15°C) for at least 24 hours, or frozen (−20°C) for at least 6 months; MPA and MPAG also were stable in urine samples subjected to three cycles of freeze and thaw. No endogenous interfering substances were seen in urine from subjects not treated with MMF.

Pharmacokinetic Analysis

Concentrations that were below the quantitation limits of 0.4, 0.1, and 4.0 mg/mL for MMF, MPA, and MPAG, respectively, in plasma, and 2.5 and 50 mg/mL for MPA and MPAG, respectively, in urine were set to zero for calculation of mean concentration and computed parameters. Concentrations of MMF and MPA were reported directly in μg/mL; all MPAG concentrations were reported in terms of μg/mL of "MPA equivalents," obtained by multiplying each MPAG concentration by 0.594 (the ratio of the molecular weight of MPA to the molecular weight of MPAG). All pharmacokinetic calculations were based on actual and not nominal sampling times. Because of secondary maxima in the plasma profiles of MPA and MPAG, calculated plasma pharmacokinetic parameters were noncompartmental, limited to half-life ($t_{1/2}$) and systemic clearance (Cl), and designated as apparent in nature.

Observed maximum plasma concentration (C_{max}) and time of maximum plasma concentration (t_{max}) of MMF, MPA, and MPAG were obtained directly from the concentration–time data. For MPA and MPAG, apparent plasma $t_{1/2}$ was calculated as $\log_e 2/k$, where k is the slope of the linear regression of the terminal portion of the log plasma concentration–time profile at 12 hours and beyond. Total area under the plasma concentration–time curve from 0 to infinity (AUC_{total}) then was calculated as $AUC_{last} + C_{last}/k$, where AUC_{last} is the area under the concentration–time profile from time zero to the last time when a quantifiable plasma concentration (C_{last}) was seen, computed using the linear trapezoidal rule. The plasma $t_{1/2}$ of MMF after intravenous administration was too short to be accurately assessed; therefore, only AUC_{last} was reported.

Apparent Cl of MPA and MPAG was calculated as the ratio of the MMF dose (expressed as dose of MPA) to the corresponding AUC_{total}. The Cl of MMF administered intravenously was calculated in two different ways: as the ratio of dose to plasma AUC_{last} of MMF, taken as an approximation of AUC_{total}; and as the ratio of drug infusion rate to plasma C_{max} of MMF, taken as an approximation of the final steady-state concentration.

Absolute bioavailability (F) was calculated for both MPA and MPAG as the ratio of AUC_{total} of oral administration to that of intravenous administration for each corresponding analyte and expressed as a percentage. Partial AUC values (AUC_{0-24} and AUC_{0-48}) were calculated for MPA and MPAG using the linear trapezoidal rule over the respective time intervals. The total amount of each analyte that had been excreted in urine over the 48-hour period (Ae_{0-48}) was calculated using the measured urine concentrations of MPA and MPAG; the respective Cl_R values were computed from these amounts as the ratio of Ae_{0-48} to the AUC of the plasma concentration profile over the same period. The Cl_R values for the time periods from 0 to 24 hours and 24 to 48 hours were computed similarly. The percentage of administered MMF excreted in urine as MPA or MPAG was computed from the respective Ae_{0-48} values by expressing the latter in milligrams of MMF and dividing by the nominal dose of MMF (i.e., 1,500 mg) multiplied by 100.

All calculations were performed using proprietary routines written for Excel version 4.0 (Microsoft, Redmond, WA). Plasma and urine concentrations of MPA and MPAG and the values of the computed parameters were analyzed using the GLM procedure of the Statistical Analysis System, Version 6.08 (SAS Institute Inc, Cary, NC). An analysis of variance (ANOVA) model appropriate for a crossover design with terms for sequence, subject within sequence, formulation, and period was used.

All quoted *P* values were derived from the ANOVA and refer to formulation differences using a two-sided *P* value of 0.05 as the criterion for statistical significance. Classic 90% confidence intervals (CI) for the difference in formulation means were computed using an error term derived from the ANOVA. Upper and lower boundaries of the CI were expressed as percentages of the intravenous values taken as the reference mean.[7] Pharmacokinetic parameters for the two formulations were considered to be statistically equivalent if the 90% CI for the ra-

tio of the parameters was contained within the 80% to 120% boundary (80–120 rule).

RESULTS

All 12 participants (11 white, 1 Asian) completed the study. Mean age for all participants was 31.4 years, mean age for the 6 men was 26.2 years (range, 20–34 years), and mean age for the 6 women was 36.7 years (range, 31–45 years). Mean weight for all subjects was 71.1 kg, mean weight for the 6 men was 78.9 kg (range, 74.4–82.4 kg), and mean weight for the 6 women was 63.4 kg (range, 56.5–70.0 kg).

The study drug was well tolerated systemically. Headache, nausea, ecchymosis, and rhinitis were the most commonly reported symptoms, and all were of mild severity. No clinically significant changes were noted in laboratory data or results of physical examinations. The MMF infusion also appeared to be well tolerated: a few participants reported tenderness or irritation at the injection site, but only one reported these symptoms to be of moderate severity; the rest reported them to be of mild severity.

Mycophenolate Mofetil in Plasma

In all subjects, MMF was below quantitation limits in plasma at all times after oral administration but was present during the intravenous infusion phase. Table I shows mean and standard deviations (SD) of the plasma concentrations of MMF up to 1 hour and 10 minutes from the start of the intravenous infusion (10 minutes after stopping the infusion), at which time MMF was below quantitation limits in all subjects. Derived pharmacokinetic parameters for MMF also are given in Table I.

Plasma concentrations of MMF reached a plateau very rapidly, such that an apparent mean steady-state concentration of approximately 3 μg/mL already had been attained by 20 minutes, the earliest sampling time after commencement of the infusion. The mean C_{max} of 3.5 μg/mL (range, 1.75–5.8 μg/mL) was observed at a mean t_{max} of 39 minutes (range, 19–60 minutes).

On cessation of the infusion, plasma concentrations of MMF declined very rapidly: 5 minutes after the infusion was stopped they were below quantitation limits in four participants but still quantifiable in the rest; 10 minutes after the infusion was stopped they were below quantitation limits in all participants. Based on this observation, the $t_{1/2}$ of MMF in plasma must be ~2 minutes.

Using AUC_{last}, mean plasma Cl of MMF was estimated to be 11.6 L/min, with a range of 7.2 to 18.7 L/min; the corresponding estimate using C_{max} was 8.5 L/min (range, 4.3–14.3 L/min). The value obtained using C_{max} would underestimate Cl, because plasma concentrations of MMF never exceeded C_{max}. Correspondingly, the clearance value that was obtained by means of AUC_{last} would somewhat overestimate Cl because the $t_{1/2}$ could not be calculated. An estimated terminal AUC portion therefore could not be included. The between-subject and within-subject variability of plasma concentrations of MMF evident at steady state may relate to its high clearance, which would accentuate the effects of sampling technique or of blood flow changes through tissues.

TABLE I

MMF Plasma Concentrations and Computed Parameters After Intravenous Administration of 1.5 g MMF in Healthy Subjects (N = 12)

	MMF Plasma Concentration (μg/mL)	Mean ± SD
Nominal time		
Pre-infusion	0	
20 min	3.20 ± 1.38	
40 min	2.58 ± 1.05	
1 hr	2.74 ± 1.64	
1 hr 5 min	0.52 ± 0.43	
1 hr 10 min	<0.4 (all subjects)	
Parameter		
AUC_{last} (μg·hr/mL)		2.35 ± 0.725
C_{max} (μg/mL)		3.5 ± 1.5
Cl_s (from AUC_{last}) (L/min)		11.6 ± 3.6
Cl_s (from C_{max}) (L/min)		8.5 ± 3.4

MMF, mycophenolate mofetil; AUC_{last}, area under the plasma concentration–time curve to the last measured time point; C_{max}, maximum observed plasma concentration; Cl_s, clearance.

Mycophenolic Acid in Plasma

Mean (SD) plasma concentrations of MPA after intravenous or oral administration of MMF are given in Table II, and a plot of the mean concentrations on a logarithmic scale is shown in Figure 1. Derived parameters are shown in Table III. Plasma MPA profiles were broadly similar after intravenous and oral administration (Figure 1). Whereas measured plasma concentrations of MPA were significantly higher ($P < 0.05$) in the first hour for the intravenous regimen (during the infusion), after oral administration they were numerically higher in the terminal part of the profile (10 hours and beyond). The latter difference was not statistically significant, however. A second-

TABLE II

Plasma Concentrations of Mycophenolic Acid (MPA) and Its Metabolite, MPAG, After Intravenous and Oral Administration of 1.5 g Mycophenolate Mofetil in Healthy Subjects (N = 12)

	Mean ±SD Plasma Concentration (μg/mL)			
	MPA		MPAG	
Nominal Time	Intravenous	Oral	Intravenous	Oral
Predose	0	0	0	0
20 min	24.9 ± 7.0	8.7 ± 7.5	6.0 ± 3.3	0.87 ± 2.05
40 min	37.8 ± 6.2	28.8 ± 11.3	15.5 ± 4.3	16.8 ± 9.5
1 hr	47.0 ± 9.2	24.1 ± 10.1	27.5 ± 7.0	27.1 ± 10.7
1 hr 5 min	42.6 ± 10.8	—	32.2 ± 8.3	—
1 hr 10 min	36.4 ± 9.6	—	33.9 ± 8.4	—
1 hr 15 min	—	23.3 ± 9.1	—	34.6 ± 10.3
1 hr 30 min	19.2 ± 6.1	16.3 ± 5.7	38.6 ± 9.2	39.9 ± 7.0
2 hr	8.7 ± 3.1	10.0 ± 5.7	36.8 ± 9.1	40.2 ± 5.3
3 hr	2.9 ± 0.76	3.0 ± 1.6	26.8 ± 8.2	32.1 ± 8.7
4 hr	1.5 ± 0.61	1.7 ± 0.83	20.3 ± 7.3	25.1 ± 7.6
6 hr	1.5 ± 1.3	1.6 ± 1.2	13.1 ± 5.6	16.3 ± 6.3
8 hr	1.6 ± 1.5	1.5 ± 1.1	10.0 ± 5.0	11.8 ± 4.8
10 hr	1.7 ± 0.94	2.25 ± 0.79	9.0 ± 5.2	11.3 ± 3.6
12 hr	1.8 ± 0.70	2.0 ± 0.63	9.5 ± 3.5	12.2 ± 4.0
16 hr	1.1 ± 0.36	1.3 ± 0.60	8.5 ± 2.4	9.2 ± 3.2
24 hr	0.85 ± 0.29	1.1 ± 0.41	4.7 ± 1.3	6.0 ± 2.0
36 hr	0.52 ± 0.34	0.55 ± 0.25	3.1 ± 1.1	3.7 ± 1.7
48 hr	0.32 ± 0.17	0.40 ± 0.16	0.68 ± 1.25	1.4 ± 1.45

MPA, mycophenolic acid; MPAG, mycophenolic acid glucuronide.

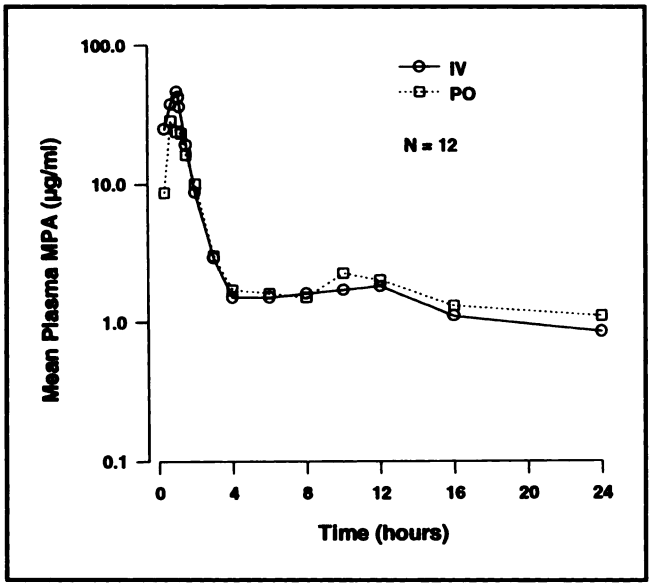

Figure 1. Mean plasma concentrations of mycophenolic acid versus time after intravenous and oral administration of a single 1.5-gram dose of mycophenolate mofetil in healthy volunteers.

ary rise in the plasma concentration of MPA occurred with both routes of administration 8 to 12 hours after the dose (Table II).

The C_{max} of MPA was 28.0% lower after oral administration than after intravenous administration ($P < 0.001$). Mean t_{max} for plasma MPA was 1 hour for intravenous administration, the same amount of time recorded for completion of the MMF infusion, and was almost identical to the mean t_{max} for oral administration. The apparent $t_{1/2}$ also was similar for both intravenous and oral administration, with a mean value of approximately 17 hours. Neither t_{max} nor $t_{1/2}$ differed significantly between the two methods of administration.

Comparison of the mean partial plasma AUCs of MPA for both means of administration showed a progressive shift in their ratio. As the time interval over which the partial AUC was calculated increased, the ratio of AUC after oral administration to AUC after intravenous administration increased toward statistically equivalent levels (Table III). The lower AUC values after oral administration relative to intravenous administration were significantly different for AUC_{0-24} ($P = 0.01$), but not for AUC_{0-48} ($P = 0.07$) or AUC_{total} ($P = 0.17$). By ANOVA, the corresponding ra-

TABLE III

Plasma Pharmacokinetic Parameters of Mycophenolic Acid After Intravenous and Oral Administration of 1.5 g Mycophenolate Mofetil in Healthy Subjects (N = 12)

Parameter	Route of Administration	
	Intravenous	Oral
C_{max} (μg/mL)	47.2 ± 9.3	34.0 ± 7.1
t_{max} (hrs)	1.0 ± 0.04	0.99 ± 0.41
$t_{1/2}$ (hrs)	16.6 ± 5.8	17.9 ± 6.5
AUC_{0-24} (μg·hr/mL)	86.2 ± 20.6	73.9 ± 14.4
AUC_{0-48} (μg·hr/mL)	99.6 ± 24.4	89.8 ± 17.8
AUC_{total} (μg·hr/mL)	108 ± 26.0	101 ± 23.4
Apparent Cl_s (mL/min)	177 ± 31	193 ± 48
F (%)	—	94.1 ± 16.2

Values are presented as the mean ± standard deviation. C_{max}, maximum observed plasma concentration; t_{max}, time to reach maximum concentration; $t_{1/2}$, apparent elimination half-life; AUC_{0-24}, area under the plasma concentration–time curve from 0 to 24 hours; AUC_{0-48}, area under the plasma concentration–time curve from 0 to 48 hours; AUC_{total}, total area under the plasma concentration–time curve; Cl_s, clearance; F, absolute bioavailability.

tios of AUC after oral administration to AUC after intravenous administration were 85.7% (90% CI, 77.4%–94.0%) for AUC_{0-24}, 90.2% (90% CI, 81.4–98.9%) for AUC_{0-48}, and 93.3% (90% CI, 85.1–101.5%) for AUC_{total}. The latter two comparisons were within the bounds for statistical equivalence.

On a within-subject basis, the mean bioavailability of MPA after oral administration of MMF was estimated to be 94.1% for the total AUC of MPA. The apparent Cl of MPA was not statistically different from the apparent oral clearance, both of which were approximately 180 mL/min. Coefficients of variation were in the range of 17% to 41% for all of these parameters except t_{max} after oral administration.

Mycophenolic Acid Glucuronide in Plasma

Mean (SD) plasma concentrations of MPAG after intravenous or oral administration of MMF are given in Table II, and a plot of the mean concentrations on a logarithmic scale is shown in Figure 2. Derived parameters are shown in Table IV. The mean plasma concentration of MPAG in the earliest sample (20 minutes after administration) was statistically significantly higher in the intravenous phase than the oral phase ($P < 0.001$). Plasma concentrations of MPAG in samples taken from 40 minutes after administration until the 2-hour time point were not significantly different for the two routes of administration, however. Beyond 2 hours, oral administration resulted in numerically higher mean plasma concentrations of MPAG at each time point except for 48 hours after administration. These differences were statistically significant ($P < 0.05$) at 3, 4, 6, 12, and 24 hours. At the 12-hour time point, mean plasma concentration of MPAG increased with both modes of administration, although the size of this effect was small.

Except for the partial AUC values, none of the derived pharmacokinetic parameters for MPAG listed in Table IV differed significantly for oral and intravenous administration. Comparison of the mean partial plasma AUCs for MPAG after oral and intravenous administration showed shifts in the ratio: in the early part of the time interval the ratio of AUC after oral administration to AUC after intravenous administration was higher than the value for the total AUC ratio (Table IV), which was opposite to the trend seen with MPA. The larger AUC values observed with oral administration relative to intravenous administration were significantly different for AUC_{0-24} ($P = 0.006$) and AUC_{0-48} ($P = 0.004$), but not for AUC_{total} ($P = 0.19$). By ANOVA, the corresponding ratio of AUC after oral administration to AUC after intravenous administration was 116.3% (90% CI, 107.8–124.8%) for AUC_{0-24}; 120.8% (90% CI, 110.9–130.8%) for AUC_{0-48}; and 108.4% (90% CI, 97.5–119.3%) for AUC_{total}. The latter comparison was within the bounds for statistical equivalence. On a within-subject basis, the mean

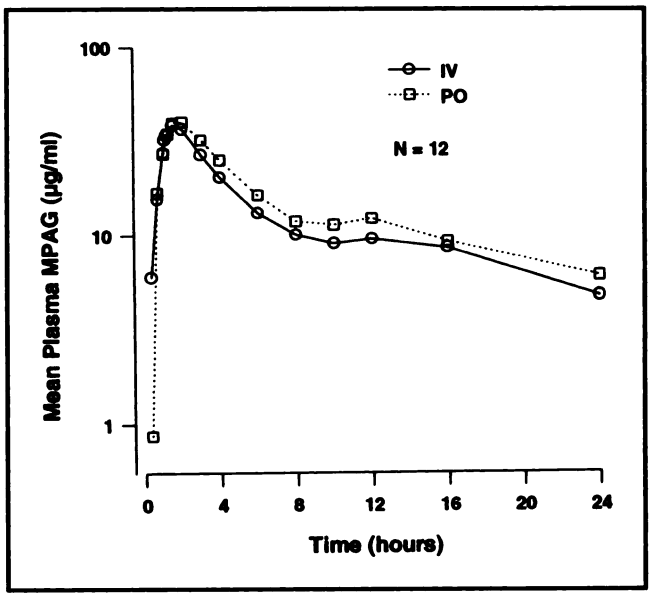

Figure 2. Mean plasma concentrations of mycophenolic acid glucuronide (MPAG) versus time after intravenous and oral administration of a single 1.5-gram dose of mycophenolate mofetil in healthy volunteers.

TABLE IV

Plasma Pharmacokinetic Parameters of MPAG After Intravenous and Oral Administration of 1.5 g Mycophenolate Mofetil in Healthy Subjects (N = 12)

Parameter	Route of Administration	
	Intravenous	Oral
C_{max} (μg/mL)	39.3 ± 9.1	43.1 ± 6.8
t_{max} (hrs)	1.65 ± 0.28	1.81 ± 0.47
$t_{1/2}$ (hrs)	21.8 ± 19.0	16.1 ± 5.2
AUC_{0-24} (μg·hr/mL)	285 ± 80.7	332 ± 75.8
AUC_{0-48} (μg·hr/mL)	340 ± 91.9	411 ± 105
AUC_{total} (μg·hr/mL)	442 ± 102	480 ± 105
Apparent Cl_s (mL/min)	44.7 ± 14.3	40 ± 8.6
F (%)	—	112 ± 25.4

Values are presented as the mean ± standard deviation. C_{max}, maximum observed plasma concentration; t_{max}, time to reach maximum concentration; $t_{1/2}$, apparent elimination half-life; AUC_{0-24}, area under the plasma concentration–time curve from 0 to 24 hours; AUC_{0-48}, area under the plasma concentration–time curve from 0 to 48 hours; AUC_{total}, total area under the plasma concentration–time curve; Cl_s, clearance; F, absolute bioavailability.

bioavailability of MPAG after oral administration of MMF was 112%, based on the total AUC (Table IV). Coefficients of variation were in the range of 15.7% to 32.1% for all of these pharmacokinetic parameters except $t_{1/2}$ after intravenous administration.

Plasma MPAG profiles broadly reflected those seen for MPA. Plasma concentrations of MPAG initially were lower than the corresponding concentrations of MPA for approximately 1 hour after administration by both routes, but subsequently always were several times higher (Table II). Mean C_{max} values for MPAG were numerically close to those for MPA, but t_{max} was longer for MPAG and approached 2 hours (Table IV). Mean apparent $t_{1/2}$ values for MPAG and MPA were similar. The mean plasma AUC_{total} of MPAG was four- to five-fold higher than that of MPA, and correspondingly, the mean apparent plasma clearance of MPAG was some four- to five-fold lower.

Mycophenolic Acid and MPAG in Urine

The amounts of MPA recovered in urine over the 48-hour period were small: concentrations of MPA were below quantitation limits in all of the interval urine collections from one participant after the oral dose and two participants (including the previous participant) after the intravenous dose. Mean total 48-hour recovery of MPA as a proportion of the administered MMF dose was 0.49% (range, 0–1.44%) after oral administration and 0.27% (range, 0–1.0%) after intravenous administration; this difference was statistically significant by ANOVA ($P = 0.02$). For both oral and intravenous administration, approximately half of the total MPA excreted over the 48-hour period was excreted in the first 24 hours. The mean total amount of MPA excreted over the 48-hour period after oral administration of MMF was 181.4% (90% CI, 127.5–235.2%) of that after intravenous dosing.

Mean Cl_R of MPA over the 0- to 48-hour interval was 1.17 mL/min (range, 0–4.6 mL/min) after oral administration and 0.56 mL/min (range, 0–2.1 mL/min) after intravenous administration; this difference was statistically significant ($P = 0.04$). Similar clearance values were obtained for the respective routes of administration over the 0- to 24-hour interval. Over the 0- to 48-hour interval, mean Cl_R of MPA after oral administration was 208.7% (90% CI, 123.8–293.5%) of that after intravenous administration.

In contrast to MPA, large amounts of MPAG were recovered in the urine, and all urine samples contained quantifiable amounts of MPAG at all intervals. In relation to the administered MMF dose, mean total 48-hour recovery of MPAG was 71.3% (SD 6.8; range, 62.5–82.9%) for oral administration and 72.1% (SD 6.65; range, 61.0–86.3%) for intravenous administration. This difference was not statistically significant ($P = 0.81$). For both oral and intravenous administration, approximately 60% of the total MPAG excreted over the 48-hour period was excreted in the first 24 hours. The mean total amount of MPAG excreted over the 48-hour period after oral administration of MMF was 98.9% (90% CI, 91.3–106.6%) of that after intravenous dosing, showing statistical equivalence of the two routes.

Mean Cl_R of MPAG over the 0- to 48-hour interval was 33.7 mL/min (SD 7.35; range, 21.7–47.2 mL/min) for oral administration and 42.5 mL/min (SD 14.9; range, 28.0–78.8 mL/min) for intravenous administration, a difference that was statistically significant ($P = 0.015$). Similar clearance values were obtained for the respective routes over the 0- to 24-hour interval. Over the 0- to 48-hour interval, mean Cl_R of MPAG after oral administration was 79.0% (90% CI, 66.0–92.0%) of that after intravenous administration.

DISCUSSION

Mycophenolate mofetil was not quantifiable in plasma at any time after oral administration. Because the limit of MMF quantitation in plasma was 0.4 μg/mL, the C_{max} of MMF after oral administration would have been no larger than this (and might have been smaller). At the infusion rate used, the same intravenous dose of MMF led to a mean C_{max} for MMF of 3.5 μg/mL (Table I), and to a mean t_{max} for MPA that was

comparable to that seen after oral administration (Table III).

Mean C_{max} of MPA after oral administration was somewhat lower than that after intravenous administration, but would have been subject to first-pass elimination. In contrast to intravenous infusion, the input rate of MMF after oral administration would not have been zero order. Judging from the early rise in plasma MPA after oral administration (Table II), peak MMF input rate after oral administration probably exceeded that after intravenous infusion. The upper limit of the absolute bioavailability of MMF after oral administration (based on the ratio of plasma C_{max} values for MMF) was therefore 0.4/3.5, or 11%.

Calculated plasma Cl of MMF was approximately 10 L/min (Table I), with a plasma $t_{1/2}$ of only a few minutes. Rapid deesterification of MMF does not occur in blood; it has a $t_{1/2}$ in isolated plasma or whole blood of several hours.[5] Therefore, erythrocyte or plasma esterases could not have been contributing appreciably to systemic clearance during the transit time to the sampling site. Comparison of the systemic plasma clearance with an expected cardiac blood output of ~5 to 6 L/min then would imply widespread metabolism of MMF in tissue, unless reversible partitioning of MMF into erythrocytes occurred that was substantially higher than plasma binding. Systemic clearance in terms of plasma then may have been several times higher than that expressed in terms of blood, but data were not available to assess this possibility.

Because MMF was measurable after intravenous administration in peripheral venous plasma from the arm contralateral to that used for the infusion, tissue in the lungs or arms was not capable of complete deesterification of MMF. Tissue esterases and endothelium with such activity are widely distributed. If the arm tissue could have deesterified MMF, sampling would have been downstream from a metabolic site, and the calculated clearance of MMF would not have represented true systemic clearance. Mixed venous and arterial blood sampling would have helped resolve some of these details. Nonetheless, irrespective of the location of the major sites of MMF deesterification, the key therapeutic conclusion that can be made from our results is that a rapid and essentially complete conversion of MMF to MPA occurred, such that systemic exposure to MMF itself was minimal after oral administration. In this respect, the morpholino ester clearly meets one of the classic desired features of a prodrug. The plasma profile of MPA after oral administration of MMF showed an early and sharp C_{max} (Figure 1), consistent with rapid absorption and rapid conversion of MMF to MPA, followed by rapid distribution and metabolism of the generated MPA.

Secondary increases in plasma MPA occurred 8 to 12 hours after administration, suggesting the possibility of enterohepatic circulation. These increases interfered with accurate calculation of terminal $t_{1/2}$. A mean apparent value of approximately 17 hours was a reasonable estimate and confirmed that twice daily administration of MMF would be appropriate clinically, because MPA is the pharmacologically active species.

The calculated plasma clearance of MPA of approximately 108 mL/min (Table III) underestimated true systemic clearance because part of the measured plasma AUC of MPA was contributed by amounts of drug recirculated by enterohepatic circulation. Because renal plasma clearance of MPA was approximately 1 mL/min, excretory removal of MPA by the kidney was an insignificant part of systemic clearance. More than 70% of the dose was recovered in urine, consistent with almost all of the MMF dose being finally excreted in urine. More than 99% of the material in urine was MPAG, indicating that systemic clearance of MPA must have occurred almost entirely through metabolism. Although the liver clearly played a major role in this metabolism, a number of other tissues, including the gut wall and kidneys, had the capacity for glucuronidation. Evidence for renal metabolism is discussed below.

The early plasma MPAG profile was a delayed and broadened version of that for MPA (Figures 1 and 2), consistent with the fact that MPA is the metabolic precursor to MPAG. The terminal portion of the profile showed that the apparent $t_{1/2}$ of MPAG was similar to that of MPA (Tables III and IV), again reflecting a precursor–successor relationship between MPA and MPAG. Assigning each particular entity to a particular role was impossible, however, because MPAG was both the precursor for MPA through enterohepatic circulation and the successor to MPA through metabolism. Renal clearance of MPAG was approximately 40 mL/min, which was not much less than plasma clearance of MPAG; primarily, this closeness occurred because renal excretion of MPAG was the final, and essentially the only, elimination pathway.

The calculated plasma clearance of MPAG would have been somewhat overestimated by the assumption that all of the MMF dose became available as plasma MPAG, because some drug substance was lost in the feces during enterohepatic circulation and some (1% in this study) was excreted in urine as MPA. Renal metabolism of MPA and subsequent direct tubular excretion of MPAG, as discussed below, would have reduced the plasma AUC of MPAG, and hence also would have increased plasma clearance somewhat. Because these effects were relatively mi-

nor and MPAG was the sole metabolite, plasma clearance of MPAG after oral administration appears to be a reasonable means of estimating true systemic clearance of MPAG, such as would have been obtained by intravenous administration of MPAG.

Comparison of the renal excretion of MPA and MPAG showed that the two species were handled differently by the kidney. Mean protein binding of MPA in plasma from healthy subjects is high, at ~97%.[8] With a normal glomerular filtration rate of approximately 120 mL/min, the calculated Cl_R of MPA would be approximately 3.6 mL/min if no filtered drug was reabsorbed. Because this is well above the observed value, renal reabsorption of MPA involving passive or active mechanisms in fact must have occurred. In contrast, mean plasma protein binding of MPAG is ~82%;[8] therefore, assuming complete excretion of the free fraction and the same glomerular filtration rate, Cl_R of MPAG would be 21.6 mL/min, well below the observed values. In addition to passive filtration clearance, active renal tubular secretion of MPAG also must have occurred.

The total plasma AUCs of MPA after oral and intravenous administration of MMF were statistically equivalent by the 80–120 rule. Oral administration of MMF in humans thus fulfills its intended prodrug function of enhancing the systemic bioavailability of MPA.[1] This represents a worthwhile gain, because clinical efficacy depends on plasma concentration of MPA, and gram doses of drug are required to achieve therapeutic concentrations of MPA.

The total plasma AUC of MPA is the sum of two major contributions, with the primary contribution derived directly from deesterification of MMF to MPA and the secondary contribution from enterohepatic circulation. The magnitude of each contribution is dependent on the route of administration. Thus, MMF administered orally is subject to a first-pass effect, so that the first component is reduced relative to the same intravenous dose. Enterohepatic circulation then partly compensates in the secondary component. Comparison of partial AUC values yielded evidence for the operation of these mechanisms: the AUC_{0-24} of MPA after oral administration was statistically significantly less than and not statistically equivalent to AUC_{0-24} of MPA after intravenous administration, whereas the subsequent AUC_{0-48} and AUC_{total} were statistically equivalent for both routes of administration. Because these processes may be altered by concurrent disease processes or physiologic abnormalities, our result of almost complete bioavailability of MPA after oral administration of MMF in healthy subjects may not hold true under all clinical conditions.

Complete absorption of MMF after oral administration is indicated if excretion of MPAG in urine over the 48-hour period is equivalent after both intravenous and oral administration. The AUCs for total plasma MPAG after oral and intravenous administration also were statistically equivalent, with a ratio of AUC after oral administration to AUC after intravenous administration of 108%. For both the partial AUCs (0 to 24 hours and 0 to 48 hours), however, the AUC of MPAG after oral administration was significantly greater than (and not statistically equivalent to) that after intravenous administration. This early difference between routes of administration in AUC of plasma MPAG suggests that metabolism of MPA may have occurred in the kidney as well as the liver,[9] although enterohepatic circulation and extensive protein binding of MPA complicate a theoretical analysis based on plasma MPAG alone.

A more direct argument for the occurrence of MPA metabolism in the kidney, based on Cl_R, follows. Because the observed difference between oral and intravenous administration in AUC of plasma MPAG over the 0- to 48-hour interval was not accompanied by a difference in renal excretion of MPAG, it follows that Cl_R of MPAG over the 0- to 48-hour interval was significantly greater after intravenous administration than after oral administration. This result did not seem to be due to nonlinear behavior, because Cl_R values for MPAG were the same for the 0- to 24-hour and the 0- to 48-hour intervals for both routes of administration, even though average concentrations of MPAG were lower overall for the 0- to 24-hour interval, particularly after oral administration (Table IV).

Glucuronidation of MPA inside renal tubular cells allows direct tubular excretion of MPAG into urine without MPAG contributing to the plasma AUC of MPAG. Based on the discussion above, such excretion must involve a tubular transport mechanism. Partial plasma AUCs of MPA were higher after intravenous administration than after oral administration. Systemic (including renal) exposure to MPA was therefore higher, generating relatively higher concentrations of MPAG within tubular cells from intracellular MPA glucuronidation. Consequently, direct excretion of MPAG into urine was higher, and the plasma AUC of MPAG was therefore lower for the same total quantity of MPAG in urine.

Although the difference between the two routes of administration in plasma concentrations of MPA was presented as the basis for the subsequent renal differences, intravenous administration also resulted in substantially higher plasma concentrations of the lipophilic species MMF. Although in principle this also could contribute to higher renal tubular cell MPA concentrations via intracellular deesterification of MMF, the relatively short time that MMF was found in plasma and the comparability of MPAG clearance during the 0- to 24-hour and 0- to 48-hour

intervals after intravenous administration indicate that this contribution must be minor. These differences were of minor significance in the healthy participants in this study, but renal metabolism of MPA could be of some significance in the clinical context. Direct confirmation of the renal metabolism of MPA in humans would help clarify this point.

These results characterize the basic pharmacokinetic parameters of MMF and its metabolites. As a prodrug of MPA, MMF shows rapid, complete absorption and conversion to the active species MPA after oral administration, with essentially complete presystemic removal of MMF. Evidence exists that subsequent metabolic clearance of MPA occurs in the kidney as well as the liver. The total true systemic clearance is appreciable, but at least part of the hepatically metabolized material undergoes enterohepatic circulation. Essentially, all of the MPA is eventually metabolized to a single metabolite, the pharmacologically inactive phenolic glucuronide MPAG. This is itself finally excreted in the urine, partly by a transport-mediated process. Between-subject variability in the pharmacokinetic parameters of MPA and MPAG was generally small.

In patient populations, multiple doses of MMF are used under abnormal and often changing pathophysiologic conditions, and the results of this study of single doses administered to healthy, fasted volunteers will not necessarily apply to other clinical situations. The pharmacokinetics of MMF appear to be relatively clear cut, however, encouraging the hope that its clinical use will be reasonably predictable.

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