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Report

Bioavailability Improvement of Mycophenolic Acid Through Amino Ester Derivatization

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The potential bioavailability improvement of mycophenolic acid (MPA), 1, through ester derivatization was evaluated in monkeys at a dose of 20 mg/kg in this study. The acetyl solketal ester 3 was found to have excellent partition properties but poor aqueous solubility. Thus, even though it can be converted rapidly to MPA by plasma and liver enzymes, it showed poor oral bioavailability (56% of MPA) in monkeys. The bioavailability of the morpholinoethyl ester 4 and the acetyl morpholinoethyl ester 5, on the other hand, was found to be 236 and 150% that of MPA, respectively. Since ester 5 has greater aqueous solubility, but similar chemical stability and enzymatic hydrolysis rates compared to ester 4, the better bioavailability of ester 4 may result from its greater partitioning into the gastrointestinal membranes.

KEY WORDS: Bioavailability; mycophenolic acid; ester derivatization.

INTRODUCTION

Since its isolation in 1896 (1), mycophenolic acid (MPA) (1) has been shown to have antitumor (2), antiviral (3), antipsoriatic (4), immunosuppressive (5), and antiinflammatory activities (6). Because of this broad spectrum of activities, attempts were made to increase the bioactivity or specificity of MPA by replacement of the lactone ring, modification of the aromatic ring substituents, or ester and amide derivatization (2) but failed in most cases to show improvement. Recently, the carbamate ethyl ester derivative 2 was reported to possess "stronger effects" than the parent drug on various experimental tumors (7). Compound 2, however, was found to be at least partially converted to MPA via chemical degradation before absorption could occur (8). This is due to extreme lability of the carbamate functionality at physiological pH values (9). Thus, it appears that proper derivatization could potentially improve the activity/bioavailability of MPA without sacrificing stability for absorption and for formulation development. For this reason, the acetyl solketal (3), the morpholinoethyl ester (4), and the acetyl morpholinoethyl ester (5) derivatives, which are expected to provide better stability (compared to 2) and significant differences in physical properties (compared to MPA), were prepared for evaluation and the results are reported in this paper.

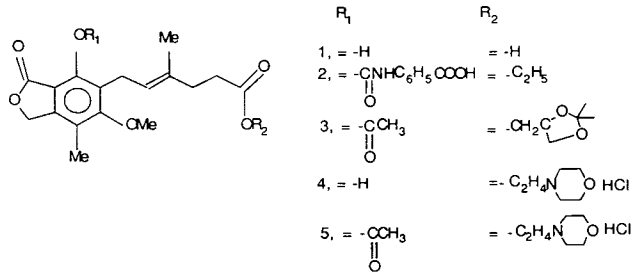
EXPERIMENTAL

Material

Mycophenolic acid (MPA) was purchased from Calbiochem. HPLC-grade acetonitrile, tetrahydrofuran, methanol, and nanopure water were used to prepare the mobile phases. All other reagents were USP or HPLC grade and were used without further purification.

Preparation of Acetyl Solketal Ester 3

MPA (5.0 g) was dissolved in dichloromethane (200 ml) and to the solution was added thionyl chloride (5.0 ml) and dimethylformamide (0.1 ml). After 8 hr the solution was evaporated under vacuum to yield the acid chloride as an oil. The acid chloride of MPA was dissolved in dichloromethane (200 ml), and pyridine (5 ml) and solketal (5.0 ml) were



Scheme 1.

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added. After 24 hr, the solution was washed with dilute hydrochloric acid, dilute aqueous sodium bicarbonate, and water. The solution was then dried over magnesium sulfate and evaporated. The resultant oil was chromatographed on silica gel (400 g), eluting with hexane:acetone 3:1, to yield the solketal ester of MPA (3.6 g) as an oil. NMR ($CDCl_3$) 1.36, 1.42 [$C(CH_3)_2$], 1.83 ($CH_3C=C$), 2.16 ($ArCH_3$), 2.3–2.5 [$C(CH_2)_2$], ($ArCH_2OCO$), 5.2–5.3 (=CH), 7.75 (OH).

The solketal ester from above (1.0 g) was dissolved in pyridine (10 ml) and acetic anhydride (0.5 ml) was added. After 2 hr the solution was added to water and extracted with ethyl acetate. The extract was washed with dilute hydrochloric acid and dilute sodium bicarbonate. The solution was then dried (magnesium sulfate) and evaporated. The residue was chromatographed on silica gel (50 g), eluting with hexane:ether 1:1, to yield the acetyl solketal ester **3** (900 mg); m.p. 78–80°C. NMR ($CDCl_3$) 1.33, 1.38 [$C(CH_3)]_2$, 3.3 (doublet, J = 5 Hz, $ArCH_2C=$), 3.5–4.3 (multiplet, dioxolane H's), 3.78 ($ArOCH_3$), 5.0–5.1 (multiplet, CH=C), 5.17 ($ArCH_2O$).

Preparation of Morpholinoethyl Ester 4

The MPA acid chloride, prepared as described above from 32 g of MPA, was dissolved in dichloromethane (50 ml), and the solution was added to an ice-cold solution of morpholinoethanol (30.5 ml) in dichloromethane (250 ml). After 90 min, the solution was washed with water, dried over sodium sulfate, and evaporated to yield the morpholinoethyl ester **4** (38 g); m.p. 93–94°C. This material was dissolved in isopropanol (200 ml) and the solution was added to a solution of hydrogen chloride (10 g) in isopropanol (150 ml). The hydrochloride salt was collected by filtration and dried under vacuum; m.p. 154–155°C. NMR (DMSO-d_6) 1.83 ($CH_3C=$), 2.10 ($ArCH_3$), 2.2–2.5 [$(CH_2)_2$], 3.0–3.5 [$N(CH_2)_3$], 3.3 (doublet, J = 5 Hz, $CH_2C=$), 3.80 (OCH_3), 3.8–4.0 (CH_2OCH_2), 4.38 ($COOCH_2$), 5.15 (=CH), 5.25 (CH_2OCO).

Preparation of Acetyl Morpholinoethyl Ester 5

The morpholinoethyl ester **4** (10.0 g) was dissolved in pyridine (50 ml) and acetic anhydride (10.0 ml) was added. After 90 min the solution was poured into water and extracted with ethyl acetate. The organic extract was washed well with water, dried over magnesium sulfate, and evaporated. The resultant oil was dissolved in isopropanol (50 ml) and the solution was added to a solution of hydrogen chloride (4 g) in isopropanol (75 ml). The hydrochloride salt was collected by filtration and recrystallized from isopropanol; m.p. 70–73°C. NMR ($CDCl_3$), 1.8 ($CH_3C=$), 2.26 ($ArCH_3$), 2.40 ($OCOCH_3$), 2.2–2.5 [$(CH_2)_2$], 2.8–3.4 [$N(CH_2)_3$], 3.30 (doublet, J = 5 Hz, $CH_2=$), 3.8–4.5 (CH_2OCH_2), 4.57 ($COOCH_2$), 5.03 (=CH), 5.20 (CH_2OCO).

HPLC Methods

The HPLC system used for analyses consisted of an Altex Model 110 pump, a Kratos Model 757 spectrophotometric detector, a Micromeritics 725 automatic injector with a 50-μl loop, a Spectra-Physics 4100 integrator, and an Altex Ultrasphere reverse-phase C_8, 5 μm, 4.6 × 250-mm column. A flow rate of 1 ml/min was used and the detection wavelength was 248 nm. Two stability-specific HPLC methods were used. Method I employed a mobile phase of 60/40 methanol/(0.01 *M* ammonium dihydrogen phosphate buffer with 0.01 *M* heptane sulfonic acid sodium salt, pH adjusted to 3.0) and was used for the analysis of **3**. Method II employed a mobile phase of 25/15/60 methanol/tetrahydrofuran/(0.01 *M* ammonium dihydrogen phosphate buffer with 0.01 *M* butanesulfonic acid sodium salt, pH adjusted to 3.0) and was used to quantitate **4** and **5**. MPA was analyzed by either method.

Determination of Aqueous Solubility and pK_a

The aqueous solubility profiles of esters **3**, **4**, **5**, and MPA were determined by adding excess drug to 0.05 *M* buffer solutions (ionic strength adjusted to 0.15 *M* with KCl) in screw-capped glass test tubes. Samples were then tumbled at 24 rpm at 25°C until a constant solubility value was obtained. The saturated solutions were filtered through 0.45-μm fluoropolymer membrane (Millipore HV) filters, quantitatively diluted with the mobile phase, and analyzed by HPLC.

The pK_a's of **4**, **5**, and MPA were calculated from pH–solubility profiles (10).

Determination of Apparent Partition Coefficients (PC)

The PC of esters **3**, **4**, and **5** were determined in a 1-octanol/0.01 *M* phosphate buffer (pH 7.4) system. In a typical experiment, 1 ml of 1-octanol (saturated with 0.01 *M* phosphate buffer) containing approximately 1 mg of ester was vigorously stirred with 25 ml of 0.01 *M* phosphate buffer (saturated with 1-octanol) for 30 min. The layers were separated by centrifugation at 2500 rpm for 10 min. The 1-octanol layer, diluted 40 times with ethanol/water (48/52), and the water layer, without dilution, were analyzed by HPLC. The PC of MPA was determined at pH 2.0 (0.01 *M* HCl) and pH 7.4 in a similar manner. The recovery of the drug in both layers varied from 94 to 105%.

Kinetics in Aqueous Solution

The chemical stability of esters **3**, **4**, and **5** were examined in 0.01 *M* HCl (pH 2.0), 0.02 *M* acetate buffer (pH 5.1), and 0.02 *M* phosphate (pH 7.4) buffer. The ionic strength was maintained at 0.15 *M* by adding KCl where necessary. The pH values of the solutions were measured at 37°C. Typically, reaction samples were prepared by adding 40 μl of a 4-mg/ml drug solution in acetonitrile to 10 ml of the desired buffer solution, resulting in 0.030 to 0.037 m*M* solutions. Pretreated (steam-washed) 5-ml amber glass ampoules were loaded with a 2-ml aliquot of reaction solution, flame sealed, and stored at 37°C. At fixed times, samples were removed and stored at −20°C until assayed by HPLC. For analysis, samples were thawed and diluted 1:1 with 0.05 *M* acetate buffer (pH 3.5).

Due to the rapid degradation of **3** at pH 2.0, a modified procedure was used. Thus, a stock solution of **3** in acetonitrile was added to acetate buffer (pH 2.0) equilibrated at 37°C. At predetermined times, aliquots were removed, quenched with acetate buffer (pH 5), and immediately analyzed by HPLC. The chemical hydrolysis reactions observed

for esters 3–5 were first order and were typically followed to three half-lives.

Kinetics in Human Plasma, Monkey Plasma, and Mouse Liver Homogenate

The enzymatic hydrolysis of esters 3, 4, and 5 was studied in human and cynomolgus monkey plasma (each diluted to 80% with 0.05 *M* sodium phosphate buffer, pH 7.4, containing 1.15% KCl) and in mouse liver homogenate (33%, w/v, in phosphate buffer, pH 7.4, containing 1.15% KCl). In a typical experiment, 50 μl of a 1-mg/ml acetonitrile stock solution of the ester (0.037 m*M*) was added to 3.0 ml of 80% plasma equilibrated at 37°C. The solutions were reacted at 37°C, and at fixed times 0.25-ml aliquots were removed and added to 0.25 ml of 0°C acetonitrile for deproteinization. The suspension was agitated for 30 sec and then centrifuged at 2000 rpm for 3 min. The supernatant was diluted with an equal volume of mobile phase and analyzed by HPLC. The enzymatic hydrolysis reactions in all cases studied were apparent first order in loss of starting ester and were typically followed to at least one half-life.

Bioavailability Studies

Four male cynomolgus monkeys weighing ~6.5 kg each were fasted from 18 hr before dosing to 4 hr after dosing but were allowed free access to water at all times. Twenty milligrams of ketamine was given intramuscularly prior to dosing to facilitate handling of the animals. Doses of 20 mg/kg of mycophenolic acid and molar equivalents of compounds 3–5 were prepared in hard gelatin capsules and were stored in a desiccator. The monkeys were administrated orally a single dose of each compound once, with a washout period of 1 week between doses. A small volume of water (20 ml) was administrated following each dose. Blood samples (6 ml) were collected by venipuncture from the saphenous vein for 24 hr after dosing. Plasma was separated from the whole blood by centrifugation and transferred to polypropylene tubes. Concentrations of total MPA in plasma were determined by HPLC after incubating plasma with β-glucuronidase from bovine liver (Sigma Co.), which hydrolyzed MPA glucuronide quantitatively to free MPA. Levels of free MPA were also determined in selected plasma samples without β-glucuronidase treatment. Pharmacokinetic analysis was carried out using the trapezoidal rule.

RESULTS AND DISCUSSION

Physicochemical Properties Evaluation

The aqueous solubilities of MPA and its ester derivatives 3–5 at 25°C as a function of pH are plotted in Fig. 1. The solubility of the acetyl solketal ester 3 was found to be independent of the pH, consistent with the nonionizable nature of the compound. For MPA and the two morpholinoethyl esters, 4 and 5, the solubility profiles follow the pH–solubility relationships defined for a typical weak acid or base. Thus, at pH <5 compounds 4 and 5 had greater solubilities than MPA but poor solubilities at pH >6. The potential hydrogen bonding capability for the phenolic functional group in 4 is a possible cause for its ~10 times less aqueous

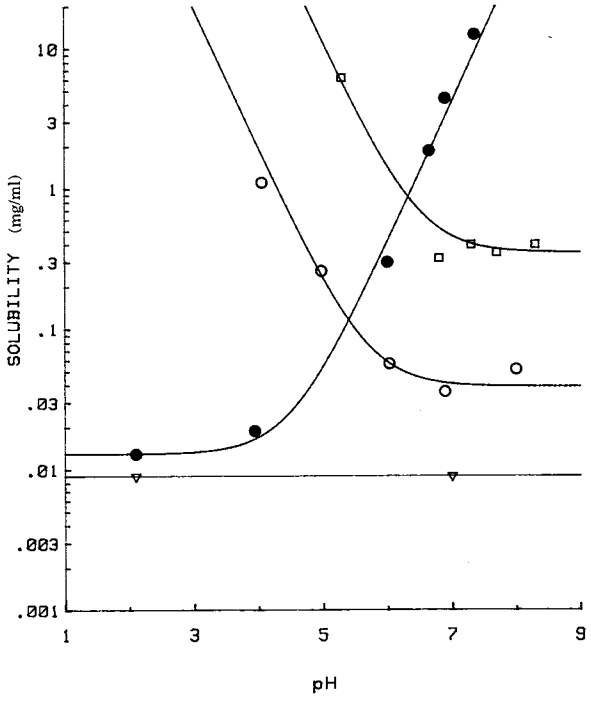

Fig. 1. Aqueous solubility of MPA (●), 3 (▽), 4 (○), and 5 (□) as a function of pH at 25°C. The lines are the theoretical curves defined by the relationships $S = S_i(1 + K_a/[H^+])$ for MPA and $S = S_i(1 + [H^+]/K_a)$ for 4 and 5. The values of S_i and K_a for each compound were taken from Table I.

solubility as compared to ester 5 (Fig. 1). The intrinsic solubility and the calculated pK_a values from the solubility profiles (see experimental) for MPA and compounds 4 and 5 are summarized in Table I. The octanol partition coefficients (PC) for these compounds were investigated at pH 7.4 and the order of increasing PC was MPA <5<4<3 (Table I).

The chemical stability of esters 3–5 was investigated at 37°C in aqueous solution at pH 2.0, 5.1, and 7.4. For each compound, MPA was the end product of hydrolysis but intermediate products were detected for compounds 3 and 5 (not shown) due to the competing hydrolysis of the two ester groups in these two compounds. Inspection of the kinetic data in Table I reveals that the two morpholinoethyl ester derivatives, 4 and 5, were reasonably stable at physiological pH values ($t_{1/2}$ > 14 hr at 37°C). Compound 3, due to the acid labile solketal functional group, was unstable at pH 2 ($t_{1/2}$ = 0.46 hr) but was stable at pH > 5 ($t_{1/2}$ > 447 hr).

In Vitro Enzymatic Conversion

The potential enzymatic conversion of compounds 3–5 to the parent drug MPA was studied first in human and monkey plasma. As was found for chemical hydrolysis, intermediate products were observed during the plasma hydrolysis of compounds 3 and 5. Since it is the overall ester conversion rate to MPA which is indicative of the availability of the esters in plasma, the time to reach 50% conversion to MPA ($t^c_{1/2}$) of each ester is reported in Table II for comparison. Ester 3 was found to be rapidly converted to MPA by either human or monkey plasma ($t^c_{1/2} \leq 49$ min). Little enzymatic hydrolysis occurred in plasma for ester 4, and the $t^c_{1/2}$ values

Table I. Physicochemical Properties of MPA and Its Ester Derivatives 3–5

Substrate	PC[a] pH 2	PC[a] pH 7.4	S_i (μg/ml)[b]	pK_a[c]	Chemical Stability, $t_{1/2}$ (hr)[d] pH 2.0	pH 5.1	pH 7.4
1 (MPA)	570	1.6	13	4.5	—	—	—
3	—	746	9	—	0.46	645	447
4	0.0085[e]	238	39	5.6	98	118	19
5	0.0039[e]	109	340	6.5	419	104	14.3

[a] *n*-Octanol–water partition coefficient.
[b] Intrinsic aqueous solubility.
[c] Calculated from the pH–solubility relationship (see Experimental).
[d] 37°C.
[e] Calculated from the Equation $pH - pK_a = \log[(PC_{int} - PC_{app})/PC_{app}]$.

in plasma (≥3.8 hr) were comparable to that in aqueous buffer ($t_{1/2}$ = 19 hr). Interestingly, ester 5 hydrolyzed rapidly in plasma ($t^c_{1/2}$ = 1.8 hr) to approximately an equal amount of MPA (through morpholinoethyl ester hydrolysis followed by acetate hydrolysis; not shown) and ester 4 (through acetate hydrolysis). The subsequent conversion of ester 4 to MPA occurred more slowly. This suggests that the enzymatic hydrolysis of the morpholinoethyl ester group is accelerated in the presence of the acetate moiety. The reasons for plasma to show such a preference, however, were not investigated.

The conversion of esters 3–5 to MPA was also examined in mouse liver homogenate. For all three esters, the conversion rate was extremely rapid at 37°C ($t^c_{1/2} \leq 3$ min, Table II) and MPA was the quantitative product.

Bioavailability Evaluation

The bioavailability of the ester derivatives 3–5 and the parent drug, MPA, was compared in cynomolgus monkeys at a 20-mg/kg dose. The major species found in plasma for all compounds were MPA and its glucoronide conjugate. No appreciable amount of esters were detected. The mean plasma concentrations (n = 4) of total MPA versus time profiles are presented in Fig. 2 and the pharmacokinetic data are summarized in Table III. Total MPA concentration (see Experimental) is used because it has been shown that the major circulating metabolites in plasma, following oral administration of MPA and its derivatives, are MPA itself and the glucuronide conjugate (metabolism occurred mostly in liver) of MPA (8,11–13). The ratio of free MPA to MPA glucuronide in plasma, on the other hand, is species and subject dependent (8,12). Therefore, to estimate the absorption characteristics of MPA and its derivatives, MPA glucuronide was converted to MPA prior to plasma assay.

Inspection of the pharmacokinetic data presented in Table III reveals ≥10-fold animal-to-animal variations for the parent drug MPA in the values of concentration maxima (C_{max}), time to reach maximum concentration (t_{max}), and area under curves (AUC). The long t_{max} of 15.0 hr also indicates that MPA is slowly absorbed in some monkeys.

Although the C_{max} values were relatively tight for the acetyl solketal ester 3, large animal-to-animal variations in t_{max} (5-fold), and AUC (3.5-fold) were also observed and the AUC value of ester 3 was only 56% of the parent drug MPA. The absorption of the two morpholinoethyl esters 4 and 5, on the other hand, was more rapid, as much shorter t_{max} values

Table II. Enzymatic Hydrolysis Data for Ester Derivatives 3–5[a]

Substrate	Plasma conversion $t_{1/2}$[b,c] Human	Plasma conversion $t_{1/2}$[b,c] Monkey	Mouse liver homogenate conversion, $t_{1/2}$[b,d]
3	10 min	49 min	<3 min
4	3.8 hr	8.5 hr	<5 sec
5	1.8 hr[e]	1.8 hr[e]	<3 min[a]

[a] 37°C.
[b] Time to reach 50% MPA conversion.
[c] Diluted to 80 vol% with phosphate buffer (pH 7.4).
[d] Diluted to 33% (w/v) with phosphate buffer (pH 7.4).
[e] Ester 5 converted to MPA and ester 4 at ~1:1 ratio at 1.8 hr. Ester 4 was then slowly converted to MPA.

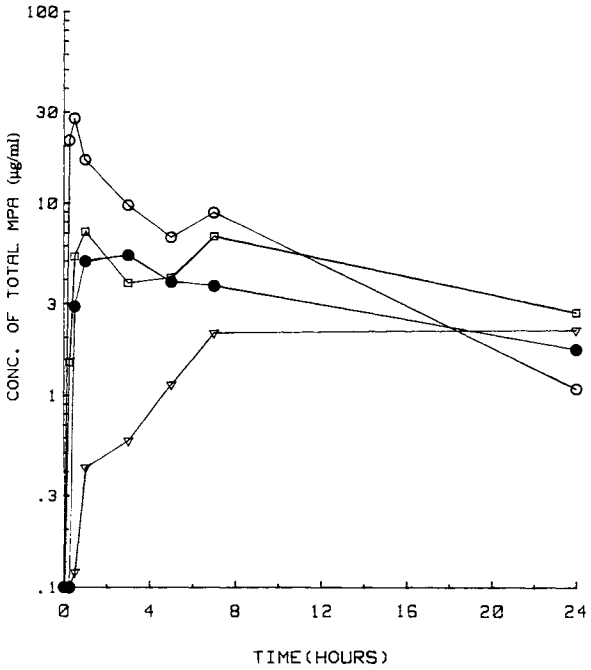

Fig. 2. Mean plasma concentrations of total MPA (see text) in cynomolgus monkeys following oral administration of single doses of MPA (●), 3 (▽), 4 (○), and 5 (□) at a dose amount equivalent to 20 mg/kg MPA.

Table III. Pharmacokinetic Parameters for Total Mycophenolic Acid in Cynomolgus Monkeys Following Oral Administration of a Single Dose (20 mg/kg)[a] of MPA or Its Ester Derivatives 3–5

Substrate	Animal ID	C_{max} (μg/ml)	t_{max} (hr)	AUC (0–24 hr) (μg/ml/hr)
1 (MPA)	A	1.2	24	17
	B	4.2	24	42
	C	12	3.0	116
	D	10	0.5	129
	Mean ± SE	6.9 ± 2.5	13 ± 6.5	76 ± 27
3	A	2.3	24	19
	B	2.6	24	52
	C	2.2	5.0	34
	D	4.3	7.0	67
	Mean ± SE	2.9 ± 0.49	15 ± 5.2	43 ± 10
4	A	66	0.5	137
	B	18	3.0	171
	C	20	0.5	167
	D	30	1.0	243
	Mean ± SE	34 ± 11	1.3 ± 0.6	179 ± 23
5	A	9.8	0.5	107
	B	7.7	7.0	107
	C	9.9	1.0	80
	D	13	1.0	162
	Mean ± SE	10 ± 0.98	2.4 ± 1.6	114 ± 17

[a] Dose amount of ester derivatives was adjusted to be molar equivalent to that of MPA.

were obtained: 1.3 and 2.4 hr, respectively. Animal-to-animal variations in the AUC values were also significantly reduced (≤2-fold) for these two compounds. Comparison of the AUC data shows that compound **4** is 1.6 times as bioavailable as compound **5**, which in turn is 1.5 times as bioavailable as the parent drug MPA.

To compare the therapeutic potential between ester **4** and ester **5**, the free MPA concentrations in plasma were also determined. Free MPA, rather than its glucuronide conjugate, is generally considered to be the active species (8,11–13). The mean plasma concentrations versus time profiles using free MPA concentrations are shown in Fig. 3 and the pharmacokinetic parameters are listed in Table IV. Thus, the t_{max} for free MPA in plasma averaged 0.7 and 2.25 hr for compounds **4** and **5**, respectively. At 7 hr after dosing, concentrations of free MPA reached a second peak (Fig. 3) for both compounds. This phenomenon may be attributed to the enterohepatic circulation of MPA via its glucuronide conjugate (8). The mean AUC values for free MPA were 83.4 and 47.4 (μg/ml/hr) for compounds **4** and **5**, respectively, indicating that the former is 1.8 times as bioavailable as the latter, consistent with the results obtained from the total MPA concentrations.

CONCLUSION

MPA has an intrinsic solubility of 13 μg/ml (Fig. 1) and a pK_a of 4.5 in water (Table I). These values are almost identical to those of naproxen (16 μg/ml and 4.4, respectively) (14), which is known to be well absorbed (~100%) in monkeys (15). Since the dissolution rate of weak acids in general is a direct function of their solubilities (16), the absorption of MPA should not be limited by its dissolution rate. The partition coefficient (PC) of MPA in its neutral form is 570 and decreased to 1.6 at pH 7.4 due to ionization. These PC values are again similar to those of naproxen (1510 and 1.2, respectively) (17). These data indicate that the bioavailability of MPA is also not absorption rate limited. Thus, the relatively poor bioavailability of MPA (43% that of **4**) may be caused by undetermined factors such as drug complexation in the GI lumen, a narrow absorption window, metabolism before absorption, etc.

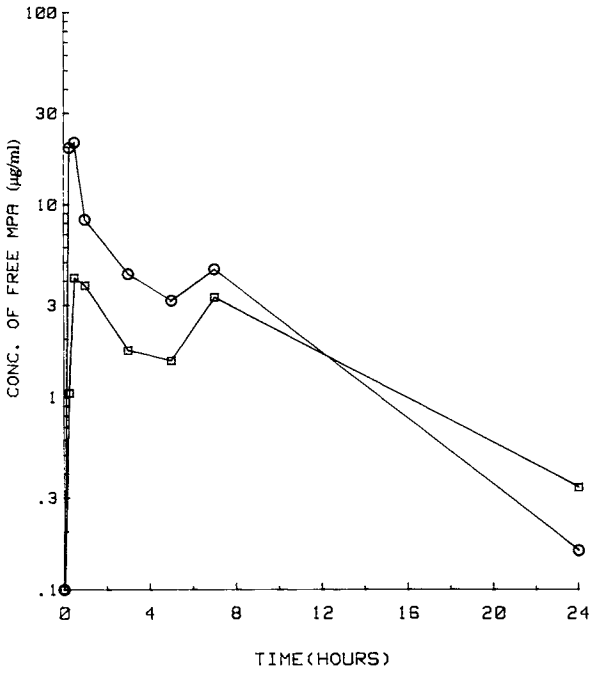

Fig. 3. Mean plasma concentrations of free MPA (see text) in cynomolgus monkeys following oral administration of a single dose of **4** (○) and **5** (□) at a dose amount equivalent to 20 mg/kg MPA.

Table IV. Pharmacokinetic Parameters for Free Mycophenolic Acid in Cynomolgus Monkeys Following Oral Administration of a Single Dose (20 mg/kg)[a] of Esters 4 and 5

Substrate	C_{max} (μg/ml)	t_{max} (hr)	AUC (0–24 hr) (μg/ml · hr)
4	22 ± 10	0.69 ± 0.17	83 ± 13
5	6.5 ± 0.67	2.3 ± 1.6	47 ± 7.2

[a] Dose amount of ester derivatives was adjusted to be molar equivalent to 20 mg/kg of MPA.

Among the three derivatives of MPA, compound 3 has the best partition property (Table I) but the poorest solubility (Fig. 1). Chemically, this compound was stable in the neutral pH region ($t_{1/2}$ > 447 hr) but degraded to its glycerol analogue (not shown) at pH 2 with a $t_{1/2}$ of 0.46 hr at 37°C. However, in view of the expected slow *in vivo* dissolution of this compound due to its extremely low aqueous solubility (<10 μg/ml, Fig. 3), the *in vivo* chemical degradation is not expected to be significant. The conversion of compound 3 to the parent MPA by plasma and liver enzymes, on the other hand, was rapid. These data suggest that the poor bioavailability of compound 3 (Table III) is a direct result of its limited solubility.

Both morpholinoethyl ester derivatives of MPA, 4 and 5, have excellent solubility at pH's <5, indicating rapid *in vivo* dissolution in the upper GI tract. Both compounds also were chemically stable at the physiological pH's of 2–7.4, indicating that they should not degrade chemically in the GI tract. The rapid appearance of MPA in monkey plasma (t_{max} ≤ 2.4 hr) relative to MPA itself (t_{max} = 13 hr) also indicates that esters 4 and 5 are absorbed intact across the GI membrane. The limited catalytic effect on the hydrolysis of these two esters in plasma, however, suggests their conversion to MPA prior to plasma appearance. Although conversion in intestinal membrane cannot be ruled out based on the current data, due to the portal circulation of intestinally absorbed drugs and the high concentrations of esterases in the liver, the most likely site for conversion of esters 4 and 5 is probably in the liver. This suggestion is supported by the rapid *in vitro* conversion of esters 4 and 5 to MPA in mouse liver homogenate (Table II) despite potential species variation. That the partition of compound 4 at physiological pH values of 2–7.4 is ~2 times better than 5 (Table I) provides a possible explanation for its greater bioavailability. The results revealed in this study promoted the further development of ester 4 (18).

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