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Pharmacokinetics and pharmacodynamics of mycophenolic acid in stable renal transplant recipients treated with low doses of mycophenolate mofetil

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Abstract Suboptimal doses of mycophenolate mofetil (MMF) are frequently employed in renal transplant (Tx) patients, with drug-related side effects or low weight. The aim of this study was to compare the mycophenolic acid (MPA) pharmacokinetic profile and its pharmacodynamic effect on patients receiving either standard (2 g) or low (1.5 g or 1 g) MMF doses, in order to evaluate the therapeutic efficacy of such low doses in inhibiting IMPDH activity. Twenty-seven stable renal Tx recipients aged 18–65 years, with a post-Tx follow-up of 38.5 ± 44.8 months (6–166 months), receiving 1 g (n = 10), 0.75 g (n = 7) and 0.5 g (n = 10) MMF twice a day in association with cyclosporine and prednisone, were included. The control group was made up of untreated healthy volunteers (n = 5). Plasma concentrations of MPA were analyzed by reverse-phase HPLC. IMPDH activity was determined in lymphocytes by the measurement of ^{3}H release from [2,8-^{3}H] hypoxantine. The mean value of areas under the concentration-time curves (AUC_{0-12}) of MPA throughout the 12-h dosing interval in patients treated with 2 g was higher than the corresponding data in patients receiving 1.5 g or 1 g bid, but no statistical differences were observed between the three groups. There was no correlation between MPA-AUC_{0-12} values and MMF dose (expressed in g/day or g/kg per day). Predose MPA concentrations correlated only weakly with the respective MPA-AUC_{0-12} values (r^2 from 0.385 to 0.655), whereas an acceptable correlation was observed between MPA C_{max} and MPA-AUC_{0-12} (r^2 from 0.626 to 0.759) in 2 g, 1.5 g, and 1 g MMF groups. An inverse relationship between MPA concentrations and IMPDH activity was observed. In general, the maximum MPA concentration was achieved from 1 h to 2 h after dosing, and the maximum inhibition of IMPDH was also from 1 h to 2 h after dosing. The evaluation of IMPDH activity demonstrated that there was a significant statistical difference between samples from 0 to 1 h (P = 0.008) and 0 to 2 h (P = 0.04). In conclusion, concentration-time profiles of renal transplant recipients administered 0.75 g and 0.5 g twice a day are slightly lower than those from the 2 g group, but nor significantly. On the other hand, inhibition of IMPDH activity was comparable in the three groups, indicating considerable interindividual pharmacodynamic variability. Pharmacodynamic monitoring of the degree of immunosuppression and its correlation with MPA plasma concentrations will be assessed further in future studies.

Key words Mycophenolic acid · Pharmacokinetics · Pharmacodynamics · Kidney transplant

Introduction

Mycophenolic acid, the active metabolite of the prodrug mycophenolate mofetil (MMF), is a potent and specific inhibitor of de novo purine synthesis in lymphocytes. This inmunosuppressive agent blocks de novo DNA synthesis and lymphocyte proliferation by inhibiting inosine monophosphate dehydrogenase type II (IMPDH II), the key enzyme in GTP and dGTP biosynthesis [1]. Results from several clinical trials have shown that the introduction of MMF to transplant therapy regimens, based on cyclosporine or tacrolimus, will provide a significant reduction in the early acute rejection rate [13].

Therapeutic drug monitoring of immunosuppressive agents is based on the measurement of drug concentrations. The utility of therapeutic drug monitoring of MPA in clinical transplantation has been evaluated in some clinical trials, the preliminary results of which permit the establishment of a therapeutic range for C_{min} from 2 μg/ml to 4 μg/ml [5, 11]. Concerning AUC, the results show a good correlation between AUC value and outcome of patients. MPA AUCs from 40 μg.h/ml to 65 μg.h/ml have been observed in patients free of kidney rejection [10, 12]. Nevertheless, traditional pharmacokinetic profile monitoring of MPA as a guide for dosage could be difficult, particularly in patients treated with multiple immunosuppressive drugs. Pharmacodynamic monitoring of the degree of immunosuppression produced by this immunosuppressive agent opens up the possibility to improve drug therapy in transplant recipients. This alternative approach involves the measurement of the degree of inhibition of the enzyme IMPDH in peripheral blood lymphocytes (whole blood), and its correlation with MPA plasma concentrations [6, 7].

Results from multicenter studies showed a decreased incidence of acute rejection episodes in patients with steady state MPA AUC_{0-12} plasma levels > 40 μg.h/ml. In those patients treated with suboptimal doses due to adverse events, mainly hemathological/gastrointestinal, and disorders, MPA concentrations should be monitored to clarify its therapeutic efficacy.The aim of this study is to evaluate the correlation between MPA concentrations and IMPDH activity in renal transplant recipients treated with either standard (1 g bid) or low MMF doses (0.75 g bid and 0.5 g bid), in order to assess whether low doses of this immunosuppressive agent may provide therapeutic plasma concentrations of MPA and adequate immunosuppression.

Materials and methods

Patients and specimens

Twenty-seven stable renal transplant recipients receiving 1 g ($n = 10$), 0.75 g ($n = 7$) and 0.5 g ($n = 10$) of MMF twice a day were involved in the study. MMF therapy period of time ranged from 6 to 14 months. Immunosuppressive drug treatment also consisted of CsA and prednisone. The mean age was 42.5 ± 13.6 years (ranging: 18–65 years) with a post-Tx follow-up of 38.5 ± 44.8 months (6–166 months).

For MPA pharmacokinetic profiles, blood samples (EDTA) were collected at 0, 20, 40, 75 min and at 2, 4, 6, 8, and 12 h after the MMF morning dose. The linear trapezoidal rule was used to calculate area under the curve values (AUC_{0-12}) over the 12-h dosing interval.

To investigate the relationship between plasma MPA concentrations and their byological effect, inhibition of IMPDH activity was measured in isolated lymphocytes from blood samples at times 0 (pre-dose) and 1, 2, and 4 h after dosing.

Source of drugs

Standards of MPA (M-5255) and carbamazepine (C-4024) were obtained from Sigma-Aldrich (Madrid, España). Methanol (Me 0306), acetonitrile HPLC grade (Ac 0329) and glacial acetic acid (345) were purchased from Scharlau (La Jota, Barcelona, Spain). Trichloroacetic acid and and phosphoric acid (573) were purchased from Merck. [2,8-^{3}H] Hypoxanthine was purchased from Moravek Biochemicals, Inc. (California, USA). Activated charcoal and thrombin were obtained from Sigma Chemical (St Louis, Mo., USA). RPMI-1640 culture medium was from Bio-Whittaker and OptiPhase "HiSafe" 2 from Wallac Scintillation products. Emit® Mycophenolic Acid Assay was from Dade-Behring (Behring, Marburg, Germany) and C-18 solid-phase extraction column from Supelco (Supelco®, Bellefonte, USA).

Methods

MPA measurements. Pharmacokinetic studies

Plasma concentrations of MPA were analyzed by a validated and previously reported HPLC method [3]. Briefly, to 0.5 ml of human plasma was added 100 μl of carbamazepine solution (20 μg/ml in methanol) as an internal standard, and 2 ml of 0.06 mol/l HCl. The mixture was vortexed and loaded in a previously conditioned C-18 solid-phase extraction column. The sample was washed with 1 ml of water and eluted with 1 ml of elution reagent (80% V/V methanol in 0.1 mol/l acetate buffer, pH 4.0) [8].

Chromatographic analysis of MPA and the internal standard was achieved with a C-18 Novapak HPLC column (4.6 mm x 25 cm; Waters Milford, Mass., USA) connected to a reverse Phase Micro-guard column (Waters Milford). Chromatography was carried out at 250 C with a flow rate of 1.0 ml/min, and monitored at a UV wavelength of 254 nm. The isocratic mobile phase was 0.05% aqueous phosphoric acid: acetonitrile at a ratio of 55:45% [9].

The total run time was 12 min. The working range for MPA was 0.1–50 μg/ml. Within-run and between-run variability ranged from 4.5% to 9.7%. The concentration of MPA was determined by the ratio of its peak height in relation to that of the internal standard.

Isolation and purification of lymphocytes

Human peripheral blood mononuclear cells (PBMC) were obtained from the mononuclear cell layer of the Ficoll-Hypaque gradient. PBMC were washed twice with PBS and resuspended with 5 ml of RPMI-1640 containing 10% heat-inactivated fetal calf se-

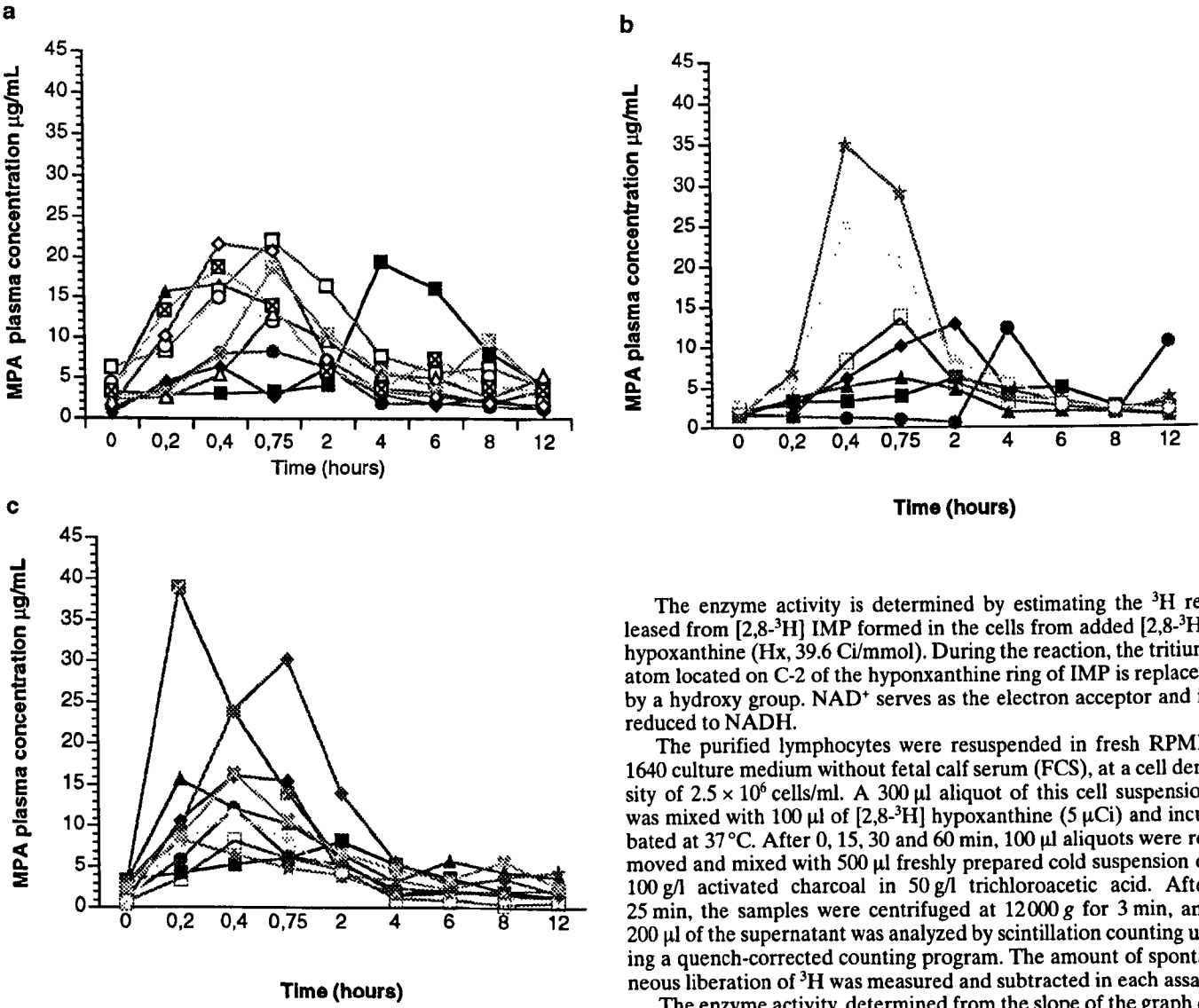

Fig. 1 Individual plasma concentrations of mycophenolic acid (*MPA*) versus time (12-h dosing interval) in 27 renal transplant recipients receiving 2 g (**a**, $n = 10$), 1.5 g (**b**, $n = 7$), and 1 g (**c**, $n = 10$)

rum (FCS). The platelets, monocytes and polymorphonuclear cells were removed by incubation with thrombin (1 U/ml) in a 5 ml disposable syringe containing 200 mg of nylon wool, during 30 min at 37 °C in 5 % CO_2 and 95 % humidified air.

The non-adherent cells were collected and centrifuged for 10 min at 1800 rpm. The remaining red blood cells were lysed by a short incubation in water followed by addition of an equal volume of PBS × 2. The purity of the lymphocytes was > 90 %.

Determination of IMPDH activity in isolated lymphocytes

Human peripheral blood mononuclear cells (PBMC) were obtained from the mononuclear cell layer of the Ficoll-Hypaque gradient. The IMPDH activity of intact lymphocytes was measured as previously described by Balzarini et al. [2] with minor modifications.

The enzyme activity is determined by estimating the ^3H released from [2,8-^3H] IMP formed in the cells from added [2,8-^3H] hypoxanthine (Hx, 39.6 Ci/mmol). During the reaction, the tritium atom located on C-2 of the hyponxanthine ring of IMP is replaced by a hydroxy group. NAD^+ serves as the electron acceptor and is reduced to NADH.

The purified lymphocytes were resuspended in fresh RPMI-1640 culture medium without fetal calf serum (FCS), at a cell density of 2.5×10^6 cells/ml. A 300 µl aliquot of this cell suspension was mixed with 100 µl of [2,8-^3H] hypoxanthine (5 µCi) and incubated at 37 °C. After 0, 15, 30 and 60 min, 100 µl aliquots were removed and mixed with 500 µl freshly prepared cold suspension of 100 g/l activated charcoal in 50 g/l trichloroacetic acid. After 25 min, the samples were centrifuged at 12000 *g* for 3 min, and 200 µl of the supernatant was analyzed by scintillation counting using a quench-corrected counting program. The amount of spontaneous liberation of ^3H was measured and subtracted in each assay.

The enzyme activity, determined from the slope of the graph of ^3H release versus time, was expressed as disintegrations per minute per minute (dpm/min). The intra- and inter-assay variability ranged from 5.1 % to 9.7 %. The correlation coefficient of the linear regression curves was > 0.90.

Statistical analysis

Data were tested for normal distribution with the Kolmogorov-Smirnov test. Correlation between variables were carried out by univariate linear regression analysis for comparison between two non-normally distributed groups, non-parametric analysis (Mann-Whitney U-test) was applied. For comparison between two normally distributed groups, an unpaired or paired (two-tailed) test was used.

Results

The pharmacokinetic data for MPA showed a high inter-individual variability even if patients were treated with the same dose of MMF (Fig. 1). A small second peak of

Table 1 Pharmacokinetic and pharmacodynamic parameters of MPA in kidney transplant recipients treated with different doses of MMF. Data are mean ± SEM and median (range). C_{min}, minimum concentration of MPA; C_{max}, maximum concentration of MPA; T_{max}, time to maximum concentration; AUC_{0-12}, area under the curve from 0 to 12 h

Group	C_{min} (μg/ml)	C_{max} (μg/ml)	T_{max} (h)	AUC_{0-12} (μg · h/ml)	Dose (mg/kg per day)	IMPDH activitiy inhibition (%)	
						1 h	2 h
2 g MMF/day (n = 10)	2.06 ± 1.20	11.97 ± 5.73	1.37 ± 1.02	49.80 ± 24.80	30.31 ± 7.94	41.53 ± 26.60	49.38 ± 45.17
	2.03	10.95	1.25	46.43	30.09	48.0	17.0
	0.53–4.4	4.3–23.7	0.67–4.0	22.5–97.5	21.28–49.5	3.8–80.7	16–100
1.5 g MMF/day (n = 7)	1.38 ± 0.62	14.09 ± 11.71	1.58 ± 1.16	41.74 ± 13.24	22.65 ± 2.33	41.00 ± 30.03	50.10 ± 31.17
	1.40	10.80	1.25	41.10	23.08	41.9	42.9
	0.64–2.4	4.1–36.8	0.67–4.0	24.2–58.5	18.99–26.32	9.2–73.8	6.6–84.3
1 g MMF/day (n = 10)	1.76 ± 0.70	16.22 ± 12.49	0.93 ± 0.62	42.87 ± 17.86	17.92 ± 4.67	67.54 ± 32.73	81.95 ± 27.96
	1.63	11.40	0.67	37.95	16.67	61.20	88.6
	0.99–2.9	5.56–46.2	0.33–2.0	25.6–80.99	14.71–30.3	5.4–100	16.2–100

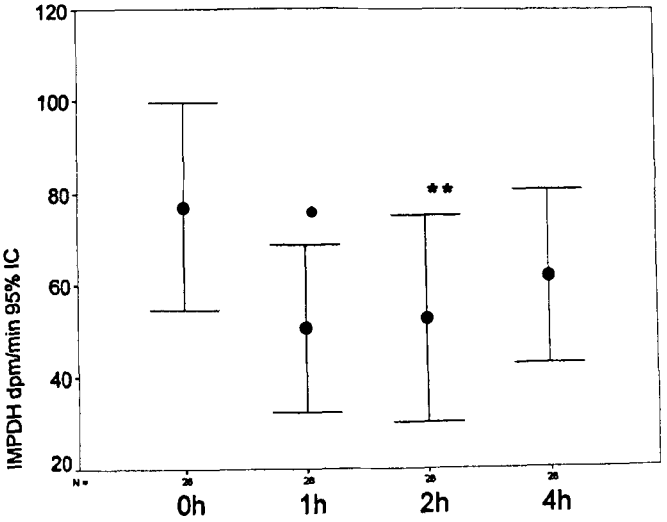

Fig. 2 IMPDH activity in MMF 1–2 g/day

MPA in plasma was observed in some patients between 6 and 12 h after dosing.

The median and the mean values ± SD for C_{min}, C_{max}, T_{max} and MPA-AUC_{0-12} obtained by HPLC from the three renal transplant groups receiving different doses of MMF are shown in Table 1. The pharmacokinetic data parameters are comparable to those reported previously in stable adult renal recipients [4]; no statistical differences were found between the three groups. The results showed a weak correlation between C_{min}/MPA-AUC_{0-12} (r^2 from 0.385 to 0.655) and an acceptable correlation between C_{max}/MPA-AUC_{0-12} (r^2 from 0.626 to 0.759). There were no correlations between Dose/Cmin and Dose/MPA-AUC_{0-12} (Dose: g/day or g/kg per day).

With regard to the effect of MPA concentrations on IMPDH activity, for the majority of the patients an inverse relationship between MPA levels and IMPDH activity was observed. The peak MPA concentration was achieved at approximately 1–2 h after dosing and the maximum inhibition of IMPDH was also from 1 to 2 h after dosing (Table 1). On the other hand, as long as the MPA plasma concentration decreases throughout the dosing interval, IMPDH activity is being restored (Fig. 2).

Four patients out of sixwith MPA-AUC_{0-12} mean values lower than 30 μg.h/ml (3 × 2 g, 1 × 1.5 g, and 2 × 1 g) achieved IMPDH inhibition comparable with those patients who had MPA-AUC_{0-12} values > 40 μg.h/ml. Nevertheless, two of these six patients, with MPA C_{max} values of 4.7 μg/ml and 8.4 μg/ml, showed weak inhibition in IMPDH activity (about 23%).

The evaluation of the IMPDH activity throughout the dosing interval demonstrated that there was a significant statistical difference between samples from 0 to 1 h (P = 0.008) and 0 to 2 h (P = 0.04). On the other hand, there was no significant statistical difference in IMPDH activity between samples from 0 to 4 h (Fig. 2). The profile of IMPDH activity is comparable in the three groups receiving different doses, and no significant statistical differences were observed. In untreated healthy volunteers IMPDH activity was stable during a 4-h time course.

Discussion

Patients receiving 2 g of MMF per day (mean dose, 0.030 ± 0.008 g/kg per day) have MPA-AUC_{0-12} and predose MPA concentrations higher than those in patients treated with 1.5 g (mean dose, 0.022 ± 0.002 g/kg per or 1 g (mean dose, 0.017 ± 0.004 g/kg per MMF, but no statistical differences were observed among the three groups.

These results demonstrate high pharmacokinetic variability in stable renal recipients that could be explained by the lack of correlation between Dose (g/day or g/kg per day) and MPA plasma concentrations. In fact, four out of ten patients administered 1 g/day

show a MPA-AUC_{0-12} profile comparable to the majority (seven out of ten) of those treated with 2 g day of MMF.

Six (3 × 2 g, 1 × 1.5 g, and 2 × 1 g) out of 27 patients (22.2%) showed an MPA-AUC_{0-12} value < 30 μg.h/ml (lowest value, 22.5 μg.h/ml), whereas seven (5 × 1 g, 2 × 1.5 g) out of 27 patients (25.9%) showed an MPA-AUC_{0-12} value < 40 μg.h/ml. Fourteen patients had MPA-AUC_{0-12} values from 40 to 97.5 μg.h/ml. On the other hand, all of these patients showed and stable renal function free of rejection episodes.

The small second peak of MPA in plasma that is observed in some patients between 6 and 12 h after dosing, could explain the high interindividual pharmacokinetic variability, and is most likely due to enterohepatic circulation of MPA from its metabolite MPAG.

Concerning inhibition of IMPDH activity, a considerable interindividual variability could be observed. Patients with similar plasma MPA predose concentrations, and also with comparable MPA-AUC_{0-12} values, have different degrees of IMPDH inhibition. These findings suggest the important role that Pharmacodynamic monitoring could play in the improvement of individual immunosuppressive therapy.

The IMPDH activvity profile during a 4-h time postdose administration was similar to that reported in previous studies [6], with a peak of inhibition ranging from 41.9% to 88.6% (median value).

The clinical utility of the measurement of IMPDH activity in lymphocytes will be further assessed in future studies involving more specific transplant patients groups under MMF therapy.

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