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Pharmacokinetic monitoring of mycophenolate mofetil in kidney transplanted patients

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Abstract

Mycophenolate mofetil (MMF) is a new immunosuppressant drug used in association with cyclosporin and oral corticosteroids to prevent acute rejection following renal allograft transplantation. MMF is an ester pro-drug of mycophenolic acid (MFA), the true active species, into which it is completely transformed after oral administration. The recommended initial dose to prevent kidney transplant rejection is 2 g/day irrespective of body weight, 1 g twice daily. The goal of this study was to correlate dosage (fixed or by body weight) and toxic effects to some non-compartmental values such as peak level (C_{max}), time to peak level (T_{max}) and trough level (C_{min}). In a small number of patients who had already reached the plasma steady state, we found a large inter-patient variability, while the same qualitative pharmacokinetic profile (as T_{max}) was conserved. At plasma trough level > 4 μg/ml some serious toxic effects were observed, whereas at $C_{min} < 2$ μg/ml, there were some cases of interstitial rejection. There was also a negative correlation between dosage and body weight, suggesting that dosages related to body weight might be better than fixed ones. Finally, monitoring plasma level of drug from transplantation to at least 12 months after surgery, at fixed MFA dosage, a small but significant decline of MFA plasma levels was found. © 2000 Elsevier Science S.A. All rights reserved.

Keywords: Mycophenolate mofetil; Pharmacokinetics; Immunosuppressant

1. Introduction

Mycophenolate mofetil (MMF) is the 2,4-morpholino ester pro-drug of mycophenolic acid (MPA), a natural compound isolated over 50 years ago from a *Penicillium* species [1–3]. MPA is a potent, selective, reversible and non-competitive inhibitor of inosine monophosphate dehydrogenase (IMPDH). It blocks the de novo synthesis of guanosine nucleotide which is required as a building block for DNA and RNA synthesis [4,5]. The MPA has stronger cytostatic effect on lymphocytes than on other cells, since the de novo synthesis of purines is essential for the proliferation of T and B lymphocytes, unlike other types of cells, that can use the salvage pathway for the generation of these compounds. The consequence is the block of antibody synthesis by lymphocytes B and the generation of cytotoxic T cells. The MMF has been shown to prevent rejection in several animal transplant models [6], and even to be capable of reversing ongoing rejection [7]. MFA has been shown efficacious in preventing acute rejection following renal allograft transplantation, administered in association with cyclosporin and oral corticosteroids [8–10]. Following oral administration, MMF is rapidly and completely absorbed, and then rapidly and almost completely converted by a presystemic metabolic process (plasmatic esterase) to MFA, the active immunosupressant species [3,10,11]. Thus, after oral administration, MMF is sometimes below the detection limit in peripheral venous plasma, while, if the same dose is administered intravenously, it may be monitored.

The pharmacokinetics of this drug is complicated by the fact that it is firstly metabolically transformed into

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a pharmacologically inactive glucuronide (MPAG) by hepatic glucuronyl transferase [11]. MPAG undergoes enterohepatic re-circulation, enabling the drug to sustain an effective plasma level, as shown by the appearance of a secondary plasma MPA peak; 6–12 h after oral intake MPA is 97% bound to plasma proteins. After oral administration of radiolabelled MMF, 93% has been found in the urine, mainly as MPGA and 6% in the faeces [12]. The mean 'apparent' half life and plasma clearance of MFA after oral administation are 17.9 h and 11.6 L/h, respectively. The AUC and peak plasma concentration (C_{max}) of MFA are approximately 50% lower in the early post-transplant period than in stable transplant recipients [12].

The first dose of MMF should be administred within 72 h from transplantation. The recommended initial dose to prevent rejection is 2 g/day, 1 g twice daily. Although dosages of 3 g/day have been used in clinical trials, there was no overall improved efficacy over 2 g/day. However, the tolerability is better in patients receiving 2 g/day than in those receiving 3 g/day. The most common adverse effects observed were diarrhoea (31% of patients receiving 2 g/day and 36.1% of those receiving 3 g/day), vomiting ($<25\%$ of patients), leucopenia (2.3% of patients) and sepsis or opportunistic infections (18–22%) [3].

The aim of this study was to ascertain any correlation between MPA plasma concentrations in patients receiving an oral daily dose of the drug after an allograft renal transplantation, and a number of variables, such as time-course, drug dosage (fixed or per body weight), frequency of transplanted organ rejection and toxic side effects.

2. Experimental section

2.1. Materials

MPA was purchased from Sigma. Methanol (HPLC grade), acetonitrile, 85% phosphoric acid, glacial acetic acid were purchased from Merck (Milan, Italy). Sodium acetate was purchased from Sigma (Milan, Italy).

The HPLC system consisted of a Merck–Hitachi L-5000 pump and a Merck–Hitachi L-4000 UV spectrometer set at 254 nm. The analytical column was a Lichrosphere 100 (Merck, RP C18, 250×4.6 mm, 5 μm). The mobile phase consisted of acetonitrile–phosphoric acid 0.05% (40:60, v/v) and was pumped at 0.8 ml/min. The signal was fed into a Perkin–Elmer LCI-100 integrator.

Solid phase extractions were executed with Sep-Pack C18 3 cm^3, 200 mg of silica gel (Waters Chromatography, Mildford, MA, USA). Vacuum extraction system was a Vac manifold (Waters). The data were elaborated using a Shimadzu CBM-10A (Milan, Italy).

2.2. Patients and treatment plan

The therapeutic protocol entailed the oral administration of MMF [13] in a dose ranging from 500 to 2000 mg/day divided into two half-doses. The primary treatment started 72 h after surgery.

The patients also received Cyclosporin in an oral dose ranging from 5 to 10 mg/kg depending on blood concentration. Corticosteroid therapy was also started (500 mg/day) on the second day after surgery, and was reduced over time to 6–8 mg/day as maintainance therapy.

2.3. Analytical procedure

A stock solution of MPA in methanol was prepared at 1 mg/ml. The stock solution was further diluted (methanol/water, 9:1) to obtain different standard solutions from 0.5 to 200 μg/ml.

A calibration curve was obtained by mixing 100 μl of each standard solution to 400 μl of plasma from healthy volunteers. These standard calibration samples were further diluted in 1.5 ml of water and 750 μl of 0.1 M HCl. The mixture after a short stirring was extracted in solid phase; the columns, pretreated with methanol–water 1:1 under vacuum (Vac manifold), were eluted with a constant flux of 0.5 ml/min. The first eluate was eliminated and the column further eluted in methanol–Na acetate buffer, 0.1 M (80:20), pH 4. Fifty microlitres of this eluate were stirred and injected into a HPLC system to analyse the amount of MPA.

Blood samples were rapidly centrifuged and then the plasma, if not used immediately, was stored at -20°C until further investigation.

Specimens of 0.5 ml of plasma were used for analysis, after stirring and centrifugation (5 min at 5000 rpm). The samples were extracted using the procedure described for calibration and analysed by HPLC, opportunely following a reported procedure [14].

The concentration of MMF in the samples was calculated by reference to the calibration curve generated from calibration standards for each batch of clinical samples.

2.4. Pharmacokinetic design

Pharmacokinetic assays were performed on 23 patients: 18 in primary treatment (15 men and three women) aged from 13 to 58 years, and five (four men and one woman) aged from 35 to 56 years, who had received MMT instead of Azathioprine because of rescue symptoms. The blood samples were randomly withdrawn either just after the transplant or during advanced therapy.

To test trough levels, blood samples for analysis were obtained from 23 patients for a total of 78 blood

samples withdrawn 12 h after MMF administration. To define the pharmacokinetic profile, blood samples were also withdrawn 0, 1, 2, 4 and 6 h after the last administration.

To compare the mean concentrations of MPA, the Student's t-test was applied. Statistically significance was set at $t < 0.05\%$.

The trial and its pharmacokinetic amendments were approved by the Ethical Committee of the S. Giovanni Battista–Molinette hospital. All patients gave their written informed consent, as required by Italian law.

3. Results and discussion

In the calibration curve (not reported) the MPA peak areas are plotted versus the different concentrations of calibration standards; the lower detection limit of the instrument is 0.1 μg/ml. The linear calibrated MMF concentration range of the method is 0.100–4.00 μg/ml, using 0.5 ml of plasma for analysis. Correlation coefficients were generally > -0.999.

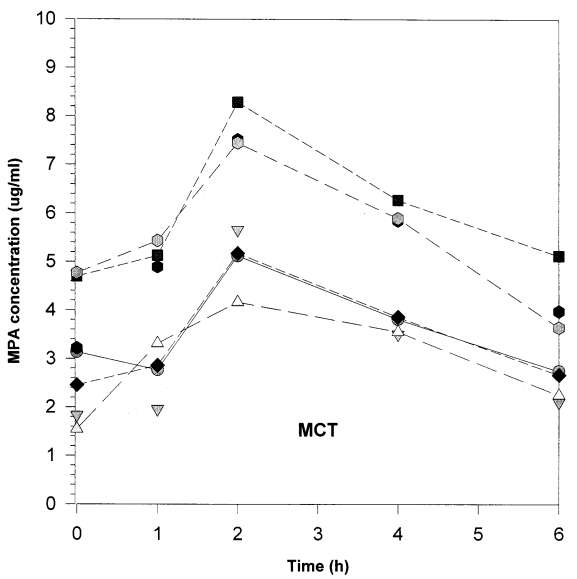

Fig. 1. Evaluation of MPA plasma concentrations. The MPA plasma concentrations from seven patients are reported; 2 μg/ml of MPA is the minimum therapeutic concentration. SD values $\leq 8\%$ of the mean values.

Table 1
Mean plasma levels of MMF with daily doses of 1000, 1500 and 2000 mg

Daily dose MMF (mg)	Samples analysed	Mean plasma levels ± SD (μg/ml)
1000	14	2.0334 ± 1.18
1500	17	2.6738 ± 1.48
2000	17	2.9576 ± 0.99

Analysis of plasma from healthy volunteers did not show any interference in retention time.

The chromatogram of MPA (not reported) from the plasma of a patient who had received 500 mg of MMF 12 h prior to the withdrawal (daily dose = 1500 mg) revealed no variation in the MPA retention time (10.5 min.). Thus, as demonstrated elsewhere [14] the HPLC method for the determination of MMF in human plasma is precise, accurate and specific.

In order to study inter-patients variability of MPA blood levels, a preliminary pharmacokinetic evaluation was performed on seven patients receiving 1000 mg/day of MMF. Plasma level monitoring was started 10 days after the start of therapy, i.e. when the plasmatic steady state had been reached [9]. Although the C_{max} plasmatic values showed a wide inter-individual variability, the qualitative pharmacokinetic profile of the drug, as shown by the value of T_{max} (peak to time) appeared to be constant (Fig. 1). C_{max} values were reached about 2 h after oral administration, as reported in the literature [11,13]. Moreover, in agreement with previous data [15] the trough level was reached 6 h after drug administration and maintained constant up to the next dose (12 h later).

These data, within the small study group, were in line with the assumption that plasma level monitoring of MPA [15] from early post-transplantation to stable transplant is not strictly necessary at present.

A further aspect to be investigated was the effects of body weight and of dosing by body weight on plasma MPA levels. At present, the literature provides few indications of correct daily oral drug dosage [3,13]. Roche, the manufacturer of the drug, suggests the oral administration of MMF 2000 mg/day, divided into two doses, starting 72 h after surgery without any dose correction on a body weight basis [13]. Moreover, the manufacturers suggest adjusting the dose only in the case of a severe renal dysfunction. From the literature [16] it is also evident that the lowest effective MPA plasma concentration to overcome rescue risk is 2 μg/ml; it is also known that concentrations above 3 μg/ml must be avoided to limit side effects [10]. Our results agree with those reported in the literature, although the minimum toxic concentration in the plasma is still uncertain. Indeed, of seven patients showing plasma MPA levels above 4 μg/ml, three presented serious side effects (piastrinopenia, leucopenia, CMV + , creatinemia). On the other hand, some episodes of interstitial rejection were observed in some transplanted patients having a trough level below 2 μg/ml.

To verify the relationship between dose and MPA plasma level, mean plasmatic concentrations were calculated for different daily drug dosages (Table 1). The difference between plasma concentrations obtained after administration of 1000 and 2000 mg/day was statistically significant, while no significant difference was

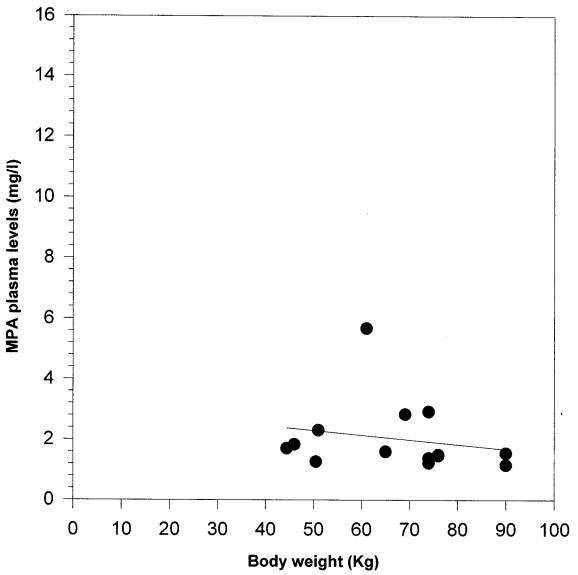

Fig. 2. Correlation between MPA dose and body weight. MPA plasma levels were monitored 12 h after administration of the drug. The patients received 1000 mg/day of MMF.

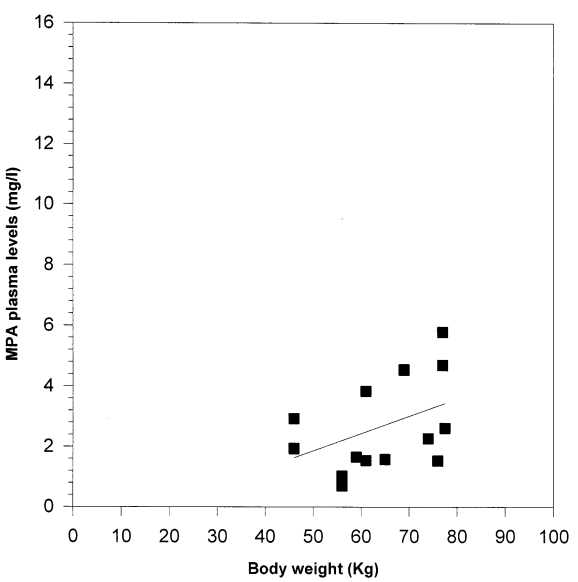

Fig. 3. Correlation between MPA dose and body weight. MPA plasma levels were monitored 12 h after administration of the drug. The patients received 1500 mg/day of MMF.

found between doses of 1000 and 1500 mg/day. Thus, only by doubling the daily dose was a statistically significant increase of plasma levels of MPA found (Table 1).

Figs. 2–4 report the relationship between trough levels and body weight of patients on different regimens of MMF (1000, 1500, 2000 mg/day). There was weak negative correlation between MPA plasma levels and body weight when 1000 or 2000 mg/day were administered, while there was no significant correlation in the case of 1500 mg/day. This result might partly be due to posology, since with the 1000 and 2000 mg/day doses, MPA was administered in two equal amounts (500 or 1000 mg) every 12 h, while with 1500 mg/day, 1000 mg was given as first dose and 500 mg after 12 h.

Fig. 5 correlates the plasma level of MPA to the administered dose of MMF per kg of body weight. Although there was a marked dispersion of data, it is clear that patients need a higher dose of MMF per kg to reach the therapeutic plasma level of MPA.

The next step was to evaluate a possible increase in the plasma level of MPA from the day immediately after transplant to 1 year later, since it has been re-

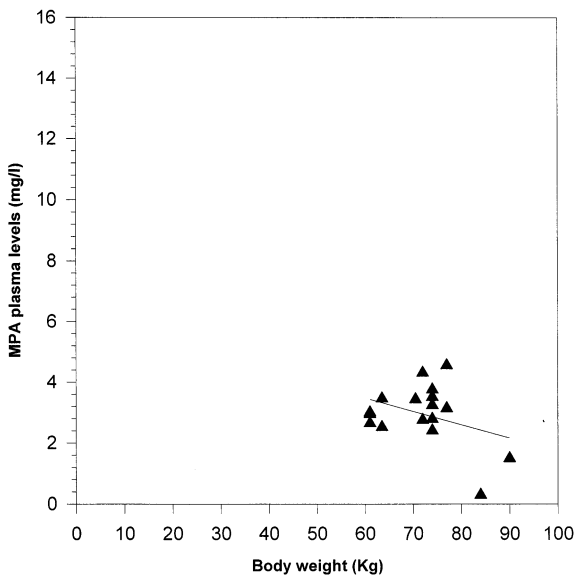

Fig. 4. Correlation between MPA dose and body weight. MPA plasma levels were monitored 12 h after administration of the drug. The patients received 2000 mg/day of MMF.

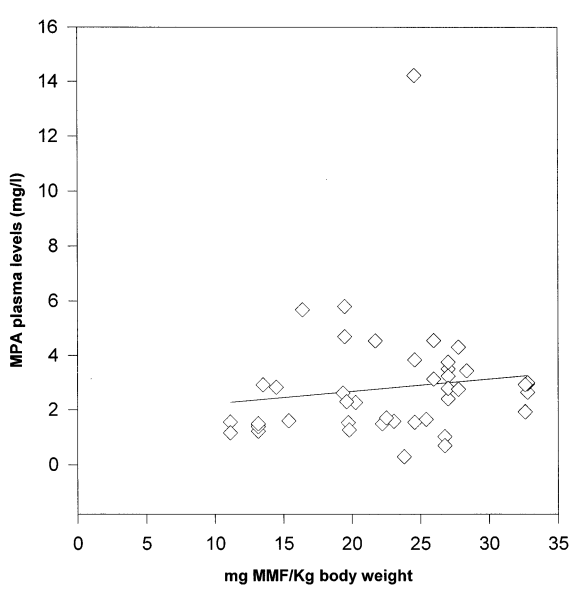

Fig. 5. Dose versus kg body weight. The plasma levels of MPA were correlated to the MMF administered dose per kg of body weight.

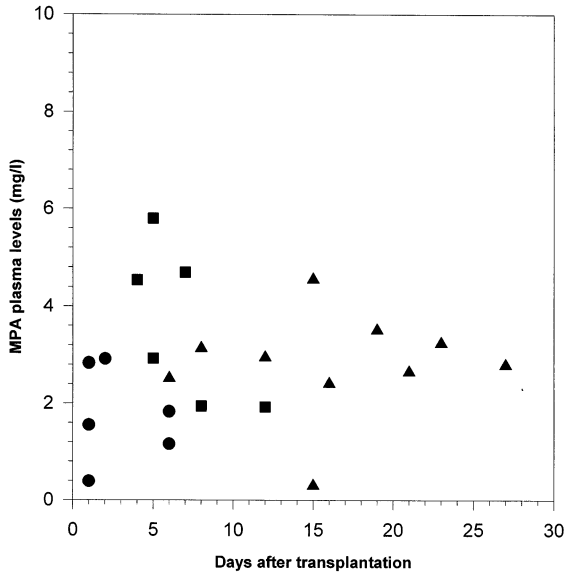

Fig. 6. Correlation between MPA plasma levels and time. MPA plasma levels were monitored 12 h after administration of the drug. Circles represent the patients who received 1000 mg/day, squares represent the patients who received 1500 mg/day, triangles represent the patients who received 2000 mg/day. SD values $\leq 10\%$ of the mean values.

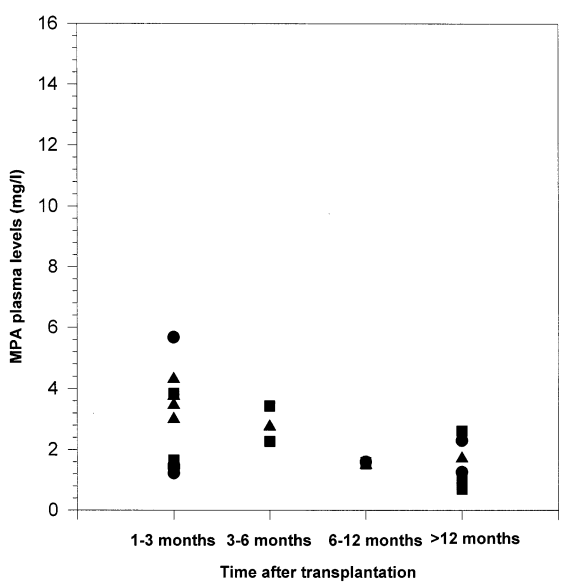

Fig. 7. Correlation between MPA plasma levels and time. MPA plasma levels were monitored 12 h after administration of the drug. Circles represent the patients who received 1000 mg/day, squares represent the patients who received 1500 mg/day, triangles represent the patients who received 2000 mg/day. SD values $\leq 9\%$ of the mean values.

ported that plasma MPA levels in the first 40 days are less than 50% of the values measured in patients with an established transplant. There is no good explanation for this; some hypotheses have been put forward, such as a change in MPA protein binding over time, or an altered enterohepatic circulation [11,15]. In particular, our study monitored plasma levels of the drug after different doses, from day 1 to day 30 after surgery, and then every 3 months.

Figs. 6 and 7 show that MPA plasma levels appear to decrease over time, even if the dosage is kept constant. More patients must be evaluated to confirm this result, which is particularly important to determine whether the effective dosage of MPA that is necessary to maintain a minimum therapeutic plasma value of 2 μg/ml must be adjusted.

Clinicians are in agreement with our hypothesis, having observed that, if a fixed dose of MMF is administered, some cases of rescue long after surgery have been seen to occur. An extended evaluation on a very large population is of course necessary to confirm this hypothesis.

In conclusion, although plasma drug monitoring of MPA is not considered to be strictly necessary to avoid rejection, blood level monitoring would be helpful to ensure a constant plasma level of the drug for a long period after surgery. Although it is common knowledge that the risk of rejection is highest in the first 3 months, long-term protocols are required that produce sufficient immunosuppression at the lowest possible dosage. Measurement of MPA levels will also provide more information about the need to administer the drug at a fixed dosage or in relation to the patient's body weight.

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