Correlation of Mycophenolic Acid Pharmacokinetic Parameters with Side Effects in Kidney Transplant Patients Treated with Mycophenolate Mofetil

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Background: Mycophenolate mofetil (MMF) is widely used in organ transplantation to prevent acute rejection. Because MMF can produce hematologic and/or gastrointestinal toxicity, therapeutic monitoring is becoming mandatory. This study was designed to investigate the relationship between the clinical events and the pharmacokinetics of mycophenolic acid (MPA) in adult renal transplantation.

Methods: Thirty-one adult kidney recipients were prospectively included in the study. MPA pharmacokinetic profiles (blood sampling at 0, 0.5, 1, 2, 4, 6, and 12 h after MMF oral dose) were obtained after transplantation (desired creatinine clearance, 40 mL/min), at 3 months after grafting, and at every clinical event (e.g., side effect or rejection). All patients received a 10-day course of anti-thymocyte globulin, cyclosporine, MMF (1 g twice daily), and steroids.

Results: We divided the 31 patients into two groups (groups 1 and 2). Ten patients (32%; group 1) had uneventful outcomes, and 21 patients (68%; group 2) presented with MPA-related side effects. For groups 1 and 2, the MPA trough concentrations (Cmin) were 1.63 ± 1.07 and 2.29 ± 1.16 mg/L, respectively (P = 0.06), and the areas under the curve (AUCs) for MPA from t0 to t12 h (MPA-AUCt0-t12) were 39.80 ± 15.29 and 62.10 ± 21.07 mg·h/L, respectively (P = 0.0005, two-sample t-test). Three patients experienced acute graft rejection after the oral MMF dose was reduced because of side effects. In this group, the MPA-Cmin and MPA-AUC were significantly lower by the time acute rejection occurred (1.00 ± 0.45 mg/L and 25.00 ± 6.20 mg·h/L, respectively). At a fixed dose (1 g twice per day), we compared the pharmacokinetic parameters of MPA [Cmin, the MPA concentration 30 min after the oral dose of MMF (C30), and AUC] according to the presence or absence of side effects in the two groups. Cmin and AUC did not differ between the two groups (Cmin = 2.22 ± 1.13 vs 2.17 ± 1.13 mg/L (P = 0.9); AUC = 66.82 ± 29.87 vs 55.70 ± 11.74 mg·h/L (P = 0.11)); and C30 was significantly higher in group 2 than in group 1 (C30 = 32.99 ± 12.59 vs 7.45 ± 5.40 mg/L; P < 0.0001).

Conclusions: Our results demonstrate a pharmacokinetic/pharmacodynamic relationship between MPA and clinical events. At a fixed dose of 2 g/day, a high C30 is associated with increased risk for side effects. This study suggests that dividing the MMF daily oral dose into more than two divided doses might prevent early MPA toxicity.

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Mycophenolate mofetil (MMF), an ester prodrug of mycophenolic acid (MPA), is widely used in organ transplantation to prevent acute rejection (1–3). MPA inhibits T- and B-lymphocyte proliferation by inhibiting inosine monophosphate dehydrogenase, thereby blocking the de novo DNA synthesis pathway in these cells and providing effective immunosuppression in transplant patients (4). MPA is converted to one active metabolite (acyl...
glucuronide M-2) and three other inactive metabolites, mainly the glucuronide metabolite (MPAG), which is eliminated by urinary excretion \((5\). Often proposed in place of azathioprine, this new regimen produces a substantial reduction of acute graft rejection after renal transplantation \((1\sim3\). MPA can cause toxicity, mainly of hematologic and/or gastrointestinal nature \((1\sim3,6\). Some pharmacokinetic/pharmacodynamic \((PK/PD\) relationships have already been reported \((6,7\). Patients with low areas under the curve for MPA \((MPA-AUC\) appear to be at high risk for experiencing graft rejection \((7\), whereas high-target AUC concentrations can increase toxicity \((6\). Considerable individual variability in pharmacokinetic \((PK\) parameters has been observed in adult and pediatric kidney recipients \((8\). In view of individual variability, toxicity, and the \(PK/PD\) relationship, therapeutic drug monitoring using different analytical methods, such as HPLC \((9\) or the Emit\(^\text{a}\) assay \((Dade-Behring)\ (10\), is becoming mandatory. To optimize clinical results, we have introduced into our practice MPA therapeutic drug monitoring using the Emit methodology, which offers the opportunity to determine both MPA and the M-2 active metabolite. With this method, the immunoassay better reflects the total immunosuppressive effect of MMF than HPLC \((11\). Our preliminary results with the Emit assay confirm the previously reported satisfactory analytical performance \((12\).

To gain insight into the safety of using MMF, this prospective study was designed to further investigate the relationship between clinical events \((side effects and rejection\) and the MPA PK parameters in adult kidney patients receiving cyclosporine-based quadruple immunosuppression in conjunction with a fixed MMF dose \((2 g/day\).

**Materials and Methods**

**Patients**

Between September 1998 and March 1999, 31 adult patients \((17 \text{ men and } 14 \text{ women}\) were prospectively included in this unicenter trial. The mean age was 43 years \((range, 16\sim67 \text{ years}\). Renal transplants were provided from cadaveric donors \((n = 28\) and from living donors \((n = 3\). Patients experiencing either severe diarrhea or active peptic ulcer, as well as patients with leukopenia \(<2.5 \times 10^3/\mu L\), thrombocytopenia \(<100 \times 10^3/\mu L\), anemia \((\text{hemoglobin} <60 g/L)\), and serologic evidence of hepatitis B surface antigen were excluded from the study. Oral informed consent was obtained in each case before enrollment in the study.

**Immunosuppression**

All patients received a quadruple immunosuppressive regimen. A 10-day course of anti-thymocyte globulin induction therapy \((ATG Fresenius, 4 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}\) was used in combination with cyclosporine \((\text{Neoral}^\text{®}, 5\sim10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}\) in two divided doses and steroids tapered down to 5 mg by 3 months. Neoral administration was controlled by cyclosporine whole-blood, trough-concentration monitoring using the AxSYM methodology \((Abbott\) and was adjusted to a target therapeutic window of \(120\sim150 \mu g/L\) during the first 3 months after transplantation and \(100\sim120 \mu g/L\) thereafter. MMF \((CellCept; Roche)\ was administrated twice daily at a fixed dose of 2 g/day, starting at the first morning after transplantation. The oral MMF dose was adjusted according to drug tolerance and related side effects.

**Study design and PK assessments**

A total of 93 MPA PK profiles were analyzed. MPA \((PK\) profiles were systematically obtained at steady state, early after transplantation, ideally when creatinine clearance reached \(40 \text{ mL/min}\), and at 3 months after transplantation. Profiles were also obtained when suspected MMF-related side effects or rejection occurred. Each PK profile consisted of blood samples \((n = 7\) obtained in EDTA tubes at trough \((predose\) concentrations \((C_{\text{min}}\) and at 30 min \((C_{30})\) and at 1 \((C_{1})\), 2, 4, 6 and 12 h after the morning MMF dose. All plasma samples were stored at \(-18 \text{°C}\) during the study period after blood centrifugation for 5 min at 2500g. Samples were analyzed retrospectively using a semiautomated immunoassay based on the Emit on the Cobas Mira Plus analyzer. Hematological side effects were defined according to the following criteria: white blood cells \(<3 \times 10^3/\mu L\), hemoglobin \(<70 g/L\), and platelets \(<100 \times 10^3/\mu L\). For upper abdominal complaints, an esophagogastroduodenoscopy was performed. In case of diarrhea, a bacteriologic examination of stools was considered. The MPA AUCs from \(t_0\) to \(t_{12}\ h\) \((\text{AUC}_{0\sim12})\) were calculated at steady state using the linear trapezoidal rule up to the last measured plasma concentration drawn 12 h after drug administration, according to the following equation:

\[
\text{AUC}_{(0\sim12)} = \frac{\sum_{i=1}^{n} (C_i + C_{i+1})}{2} \cdot (t_{i+1} - t_i)
\]

**Statistical methods**

JMP 3.2 \((\text{SAS Institute})\) and Excel 97 \((\text{Microsoft})\) were used for statistics, calculations, and graphic interfaces. To compare the \(C_{\text{min}}\) and AUC between patients experiencing and not experiencing side effects, we used the two-sided \(t\)-test for comparison of means. For other comparisons, a paired \(t\)-test was used. Outliers were eliminated with the box-plot if statistical evidence or other reasonable evidence was present. The normality of the distribution was checked with the Kolmogorov-Smirnoff test. If the normality assumption of populations could not be made \((P <0.05\) logarithmic transformation or inverse original values were used, outliers were eliminated, and normality was again verified. Even when normality could not be achieved this way, we kept the results from parametric tests. When we compared these results with those obtained with the Wilcoxon signed-rank test, no difference
could be detected. The reported P values are those of P >|t| (i.e., the probability of obtaining a greater absolute t value by chance alone if there is no difference between pairs or sample means). This is the P for observed significance of the two-tailed t-test. The alpha concentration was set at 0.05 for all analyses.

**Results**

**CLINICAL RESULTS**

The median time for the first MPA PK measurement after transplantation was 8 days (range, 3–26 days). Median creatinine clearance at the first MPA PK measurement was 59 mL/min (range, 23–78 mL/min). Because the MMF oral dose was adapted during the study according to the occurrence of side effects, the daily MMF dose (g/day) at the end of the third month after transplantation was 0–2 g/day. As shown in Table 1, 42% of patients experienced a reduction of the daily MMF dose because of side effects.

Of the 31 patients, 10 did not experience side effects. In the remaining 21 patients, 25 episodes of side effects occurred during the study evaluation up to the third posttransplantation month. Side effects included 15 episodes of leukopenia, 7 of severe anemia, 1 of diarrhea, 1 episode of esophagitis, and 1 episode of thrombocytopenia. At a fixed dose of 2 g/day, one patient presented with acute rejection (borderline according to Banff classification) with a below-therapeutic cyclosporine trough concentration (66 μg/L, as measured by the AxSYM). Three rejection episodes occurred in three other patients when the MMF dose was reduced because of side effects (leukopenia). In these patients, cyclosporine trough concentrations were within therapeutic range (mean, 116 μg/L as measured by the AxSYM).

**RELATIONSHIPS BETWEEN MPA SERUM CONCENTRATIONS AND CLINICAL EVENTS**

The mean plasma Cmin and AUC obtained from patients who demonstrated either MMF toxicity or biopsy-confirmed acute rejection and from patients who presented with no side effects are shown in Table 2. Both the Cmin and AUC were lower than in the patients with uneventful outcomes, but the number of samples did not allow statistical comparisons.

**MPA PLASMA TROUGH CONCENTRATIONS AND AUC EVOLUTION OVER TIME**

The Cmin and AUC for the early posttransplantation period and at 3 months after transplantation are compared in Table 3. Both the Cmin and AUC in patients receiving a fixed dose (1 g twice a day) displayed a progressive increase during the first 3 months after transplantation. Nevertheless, only the AUC values are statistically significantly different between these two periods (P = 0.01). This is probably because the small data set for the trough concentrations lacks sufficient statistical power.

**PK PROFILES ANALYSIS**

Individual MPA plasma concentration profiles in patients receiving 1 g of MMF twice a day are represented on Fig. 1. Individual variability occurred mainly during the early postdose period. MPA concentrations at 30 min displayed the largest variability. When individual MPA plasma-concentration profiles (0–12 h) are considered according to the occurrence of side effects, high and low C30 profiles will be obtained. Fig. 2 represents the mean MPA concentration (with SD) at each time point of the PK profiles in the groups that did or did not experience side effects. The comparison of mean Cmin, C30, and AUC according to the occurrence of side effects are represented in Table 4. As shown, the peak MPA concentration (C30) correlates better

| Table 1. Daily MMF dose (g/day) at 3 months for all patients (n = 31). |
|-------------------|---|---|
| Number of patients | Dose, g | % |
| 18 | 2 | 58 |
| 9 | 1 | 29 |
| 2 | 0.5 | 6.5 |
| 2 | 0 | 6.5 |

| Table 2. Mean (SD) Cmin and AUC0–12 h values according to clinical event. |
|-------------------|---|---|
| Cmin mg/L | AUC mg · h/L |
| Uneventful outcome | 1.63 | 39.80 |
| Acute rejection | 1.00 | 25.00 |
| Side effects | 2.29 | 62.10 |
| Acute rejection | 1.00 | 25.00 |
| Side effects | 2.29 | 62.10 |

* P = 0.0635.

**Table 3. Mean (SD) Cmin and AUC0–12 h values at early posttransplant period and at 90 days (patients receiving 1 g twice a day).**

| Cmin mg/L | AUC mg · h/L |
| Early posttransplant period | 1.94 | 44.75 |
| Three months | 2.25 | 61.28 |
| Three months | 2.25 | 61.28 |

* P, not significant.

* P = 0.01 (paired t-test).
than \( C_{\text{min}} \) and AUC with side effects in patients receiving 1 g twice a day \((P < 0.0001; \text{Table 4})\).

**CORRELATIONS BETWEEN \( C_{\text{min}}, C_{30}, C_{60}, \) AND AUC**

The correlations among \( C_{\text{min}}, C_{30}, C_{60}, \) and AUC\(_{0-12 \text{~h}}\) are shown in Fig. 3. The data used are those collected from PK profiles obtained during the entire study period, including both the early and 3-month posttransplant profiles, regardless of whether the patients received two 1-g doses a day or less. The linear correlation coefficients were 0.62, 0.77, and 0.68, respectively.

**Discussion**

MMF efficacy, as part of maintenance immunosuppressive therapy in the prevention of early acute renal graft rejection, has already been demonstrated in several multicenter studies \((1-3)\). These studies have shown the superiority of MMF (daily dose of 2 g) compared with azathioprine \((2,3)\) or placebo \((1)\) regarding positive, biopsy-confirmed acute rejection and safety when used in combination with cyclosporine. Even with an optimal dose of 2 g/day, side effects such as hematologic disorders have been observed, leading to dose reduction, with temporary drug withdrawal in some cases. The results of the current study, designed on a cyclosporine-based im-

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**Table 4. Mean (SD) \( C_{\text{min}}, C_{30}, \) and AUC according to side effects (patients receiving 1 g of MMF twice a day).**

<table>
<thead>
<tr>
<th></th>
<th>( C_{\text{min}}, \text{mg/L} )</th>
<th>( C_{30}, \text{mg/L} )</th>
<th>( \text{AUC, mg \cdot h/L} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>With side effects</td>
<td>2.22</td>
<td>32.99</td>
<td>66.82</td>
</tr>
<tr>
<td>( n = 11 )</td>
<td>(1.13)</td>
<td>(12.59)</td>
<td>(29.87)</td>
</tr>
<tr>
<td>Without side effects</td>
<td>2.17</td>
<td>7.45</td>
<td>55.70</td>
</tr>
<tr>
<td>( n = 12 )</td>
<td>(1.13)</td>
<td>(5.40)</td>
<td>(11.74)</td>
</tr>
</tbody>
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\( *P < 0.0001 \), \( *P = 0.9143 \), \( *P = 0.1114 \).

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![Fig. 1. Individual MPA plasma concentrations vs time in patients receiving 1 g of MMF twice a day.](image1)

![Fig. 2. Mean MPA concentration at each time point of the PK profiles in groups experiencing (●) and not experiencing (■) side effects. Bars, SD.](image2)
munosuppressive regimen, clearly demonstrate a PK/PD relationship between MPA concentration and side effects or acute rejection at a fixed dose of 2 g/day. A low MPA-AUC$_{0–12h}$ was associated with a high risk of rejection. These low values were obtained after a reduction in the oral dose in patients who had experienced MMF-related side effects.

Data from a Japanese study in which MMF was administered at doses of 1, 2, 3 or 4 g/day showed an increase of MPA-AUC with dose (7). In patients with low MPA-AUCs, acute rejection was more frequent than in those with high MPA-AUCs. The same study demonstrated the relatively poorer predictive value of the C$_{min}$ compared with AUC (7). In the Randomized Concentration Controlled Study (RCCT) on the safety and efficacy of MMF, the technical (13) and clinical (6) aspects of which were published recently, the authors clearly demonstrated that both the MPA-C$_{min}$ and MPA-AUC are associated with the incidence of acute rejection but not with side effects.

In our study, both the MPA-C$_{min}$ and MPA-AUC were higher in patients who presented with side effects than in those with uneventful outcomes; however, only MPA-AUC reached statistical significance. Moreover, at a fixed dose (2 g/day), the MPA concentration at 30 min (MPA-C$_{30}$) was significantly associated with an increased risk of side effects. High MPA-C$_{30}$ values would explain the occurrence of side effects in patients with MPA-AUCs within nontoxic limits. In contrast, the RCCT study showed that MMF safety is related more to the oral dose than to the MPA PK parameters [C$_{min}$, maximum concentration (C$_{max}$), and AUC]. However, it should be mentioned that in the RCCT study, there was a distinct preponderance of gastrointestinal side effects compared with other MMF-related adverse events, such as hematologic disorders. Furthermore, the withdrawal rate from the RCCT study was related to oral dose only for gastrointestinal side effects and not for all adverse events. These three trials of the safety and/or efficacy of MMF differ from each other by their design: the controlled variable in the Japanese study was the oral dose, whereas it was the target AUC in the RCCT trial and the drug tolerance at a fixed dose the current prospective study.

As indicated in experimental studies in rats (14), MMF is stable in artificial digestive fluid, whereas it is rapidly hydrolyzed to MPA by esterase in plasma and tissue homogenates. The conversion rate is higher in liver homogenates than in plasma, and is higher in plasma than in the small-intestine epithelial cells. On the basis of our results, we would suggest that the rate at which MMF is hydrolyzed by epithelial cells, plasma esterases, or both varies among individual patients and that patients in whom MMF is hydrolyzed more rapidly would thus be exposed to early toxic concentrations of MPA after oral administration of MMF. From a practical point of view, these results would postulate the beneficial effect of dividing the oral daily dose of MMF (2 g/day) into more than two doses to avoid early toxic concentrations of MPA. We think that this latter administration modality should be tested on the basis of a prospective clinical trial.

Therapeutic MMF monitoring should take into account factors that may affect MMF absorption, MPA metabolism, and the MPA free-fraction rate. The use of antacids decreases both the MPA-AUC and MPA-C$_{max}$ by decreasing MMF absorption (15). The intake of food also influences the PK of MMF, decreasing the MPA-C$_{max}$ by ~25%, without affecting MPA-AUC (15). An increase in MPA-C$_{min}$ and MPA-AUC when tacrolimus is used in conjunction with MMF has been already reported (16). In addition, the MPAG-AUC is lower in patients receiving tacrolimus in combination with MMF than in patients receiving cyclosporine in combination with MMF at the same dose of 2 g/day. Recently, it has been demonstrated that cyclosporine affects the MPA-C$_{min}$, which is lower in
patients receiving cyclosporine than in patients not receiving cyclosporine at the same MMF dose, showing less interindividual variation (17). Metabolic interactions among cyclosporine, tacrolimus, and MMF are still not clearly understood. These interactions are probably not attributable to the hydrolysis of MMF, which is extremely rapid and almost totally achieved by esterases, but could be related to a possible inhibitory effect of tacrolimus on MPA metabolism or to acceleration of MPA metabolism by cyclosporine.

Human serum albumin, high concentrations of the primary glucuronide metabolite of MPA (MPAG), and high doses of sodium salicylate significantly affect albumin MPA binding (18). Because early acute renal dysfunction after transplantation (i.e., acute tubular necrosis) is a frequent complication and renal function remains poor during the first days after grafting, a study of PK parameters is of particular interest during this period. Previous studies showed an accumulation of MPAG related to renal dysfunction (19), leading to an increase in the MPA free fraction, presumably by a competitive displacement mechanism. Special care was taken in our prospective study regarding fasting, concomitant medication, and renal function to avoid interference with determining PK parameters.

At a fixed dose (2 g/day), the MPA-AUC \(0-12\) displays a progressive significant increase during the first 3 months after transplantation, suggesting a change in the MPA PK during this period. There was also a tendency for trough concentrations to rise over time, up to the third month, but the results were not statistically significant. These changes have been described previously in adult (6) and pediatric (20) renal transplant populations and are of multifactorial origin. The increase in MPA-AUC is the result of a decrease in MPA clearance and metabolism, which is supported by the fact that MPA free fraction decreases over time (20). Factors influencing MPA metabolism may include protein binding (21), changes in enterohepatic recirculation, and induction of hepatic glucuronyl transferase activity by glucocorticoids (22), which are usually taken at high doses early after transplantation and tapered off thereafter.

In conclusion, analysis of MPA plasma concentrations in adult kidney transplant recipients treated with a fixed dose of oral MMF (2 g/day) revealed a PK/PD relationship for MPA in this prospective study of drug tolerance. Early plasma concentrations, especially at 30 min after the oral dose, seem to correlate better than MPA-AUC \(0-12\) h with side effects. These results would suggest a beneficial effect of dividing the oral dose into more than two daily doses. Moreover, additional studies are needed to confirm the role of both \(C_{\text{min}}\) and the early MPA concentration (at 30 min) in predicting the safety and efficacy in daily practice for selected patients.

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