Pharmacokinetic Basis for the Efficient and Safe Use of Low-Dose Mycophenolate Mofetil in Combination with Tacrolimus in Kidney Transplantation

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Background: Mycophenolate mofetil (MMF) is an effective posttransplantation immunosuppressive agent used in combination with cyclosporin A (CsA) or tacrolimus (Tc). An increase in plasma mycophenolic acid (MPA) has been shown in patients receiving Tc-MMF combination therapy compared with CsA-MMF combination therapy at the same dose of MMF. The aim of this prospective study was to assess the pharmacokinetic/pharmacodynamic (PK/PD) relationship for MPA in kidney transplant patients receiving low-dose MMF (500 mg twice a day) in combination with Tc.

Methods: Adult kidney transplant recipients (n = 51) were included. MPA-PK profiles (blood sampling at 0, 0.5, 1, 2, 4, 6, and 12 h after MMF oral dose) were obtained within the first 2 weeks after transplantation, 3 months after grafting, and at every adverse clinical event [side effect or acute rejection (AR)]. All patients received Tc, MMF (500 mg twice a day), and steroids.

Results: Thirty patients (59%) had uneventful outcomes, and 21 patients had 33 episodes of MPA-related side effects; only 3 patients had AR. A total of 78 MPA-PK profiles were obtained. The following PK parameters were increased in the side-effects group compared with the non-side effects group: mean MPA \(c_{\text{min}}\), 2.63 ± 1.58 vs 1.75 ± 0.82 mg/L (P = 0.016); mean \(c_{30}\), 10.47 ± 6.27 vs 7.66 ± 8.95 mg/L (P = 0.009); mean \(c_{60}\), 9.67 ± 5.42 vs 5.83 ± 2.6 mg/L (P = 0.0002); mean area under the MPA time–concentration curve from 0 to 12 h [MPA-AUC\(_{(0-12)}\)], 48.38 ± 18.5 vs 36.04 ± 10.82 mg ∙ h/L (P = 0.0006); mean dose-normalized MPA-AUC, 0.16 ± 0.05 vs 0.12 ± 0.04 (mg ∙ h/L)/(mg/m\(^2\)) (P = 0.0015). For the three AR patients, MPA concentrations obtained at the time of AR revealed MPA \(c_{\text{min}}\) values of 1.86, 1.76, and 3.83 mg/L, respectively, and MPA-AUC\(_{(0-12)}\) values of 37.7, 24.9, and 104.9 mg ∙ h/L. The threshold of toxicity was 3 mg/L (sensitivity, 38.7%; specificity, 91.5%) for \(c_{\text{min}}\), 8.09 mg/L for maximum MPA concentration during the first hour (sensitivity, 77.8%; specificity, 67.4%), and 37.6 mg ∙ h/L for MPA-AUC\(_{(0-12)}\) (sensitivity, 83.3%; specificity, 59.6%).

Conclusions: These results demonstrate the relationship between plasma MPA concentrations and toxicity. High \(c_{\text{min}}\), \(c_{30}\), and \(c_{60}\) values as well as AUC\(_{(0-12)}\) are associated with increased risk for side effects. These values may have an importance in a routine monitoring program.

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Mycophenolate mofetil (MMF) has been promising as a posttransplantation immunosuppressive agent, in combination with either cyclosporin A (CsA) or tacrolimus (Tc), following renal transplantation. MMF targets the de novo purine biosynthesis pathway by noncompetitive inhibition of inosine monophosphate dehydrogenase, acting against the proliferation of T and B lymphocytes (1). MMF

Nonstandard abbreviations: MMF, mycophenolate mofetil; CsA, cyclosporin A; Tc, tacrolimus; MPA, mycophenolic acid; MPAG, mycophenolic acid glucuronide metabolite; TDM, therapeutic drug monitoring; AUC, area under the curve; PK/PD, pharmacokinetic/pharmacodynamic; \(c_{\text{min}}\), peak concentration 30 min after oral dose; \(c_{\text{max}}\), peak concentration 60 min after oral dose; \(c_{\text{predose}}\), predose concentration; \(c_{\text{ur}}\), maximum concentration; and RCCT, Randomized Concentration Controlled Trial.
is a prodrug formulated to enhance active mycophenolic acid (MPA) bioavailability (2); it is rapidly hydrolyzed after oral administration, and peak MPA concentrations occur within the first hour after dosing (3). MPA is then converted mainly to an inactive glucuronide metabolite (MPAG), which is eliminated by urinary excretion, an acyl glucuronide (M-2), which has been shown to be active in vitro, and to two other inactive metabolites [7-O-glucoside (M-1) and M-3] (4, 5).

Initially, clinical use of MMF was distinct from other concomitant immunosuppressive drugs, such as CsA and Tc, because a therapeutic monitoring strategy was not recommended by the manufacturer. Subsequently, it appeared that MMF therapeutic drug monitoring (TDM) could optimize MMF efficacy and/or reduce side effects (6, 7). In combination with CsA, the widely used dose is 1 g (twice a day). An unexpected augmentation of MPA concentrations was observed in renal transplant patients receiving Tc and MMF in combination therapy (8), suggesting that the MMF oral dose should be reduced when these two drugs are administered concomitantly. However, no effect of MMF on Tc pharmacokinetics was observed (8, 10). Other results have shown an overlap in the individual area under the concentration–time curve (AUC) in patients receiving 1 or 2 g daily in association with Tc (9), suggesting that a dosage of 1 g/day may provide an adequate systemic concentration for achieving effective immunosuppression.

The objective of this study was to prospectively analyze the pharmacokinetic/pharmacodynamic (PK/PD) relationship for MPA in adult kidney transplant patients receiving MMF at a fixed low dose (500 mg twice a day) in combination therapy with Tc, with the goal of gaining insight into MPA therapeutic monitoring.

Materials and Methods

PATIENTS

Between October 1998 and December 1999, 51 adult patients (29 men and 22 women) were included in this single center trial. The median age was 49 years (range, 32–68 years). Renal transplants were provided from cadaver donors. Patients experiencing severe diarrhea or active peptic ulcer, as well as patients with leukopenia (<2.5 × 10^3/μL), thrombocytopenia (<100 × 10^3/μL), anemia (hemoglobin <60 g/L), and serologic evidence of hepatitis B surface antigen were excluded from the study. Informed consent was obtained in each case before enrollment in the study.

IMMUNOSUPPRESSION

All patients received a triple immunosuppressive regimen including Tc (0.2 mg · kg⁻¹ · day⁻¹) in two divided doses, MMF (Cellcept®; Roche) at a low dose (500 mg twice a day), and steroids tapered to 10 mg by 3 months. Tc administration was controlled by monitoring of whole-blood trough concentration (Tacrolimus II, IMx Analyzer; Abbott) and adjusted to a target therapeutic window of 10–20 μg/L during the first 2 weeks after transplantation and 5–15 μg/L thereafter. MMF administration was started the first morning after the transplantation. Oral MMF dose was adjusted according to drug tolerance and related side effects.

STUDY DESIGN AND PK ASSESSMENTS

MPA PK profiles were systematically obtained at steady state during the early posttransplantation period, ideally when creatinine clearance reached 40 mL/min, and at 3 months after transplantation. MPA PK 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, before dosing and at 30 min and 1, 2, 4, 6, and 12 h after the morning MMF dose (cimin, c30’, c60’, c120’, c240’, c360’, c480’, c600’, c720’, c12h). After blood was centrifuged for 5 min at 2500 g, plasma samples were obtained and stored at −18°C during the study period. The plasma samples were analyzed retrospectively by a semiautomated immunoassay based on an enzyme-multiplied immunotechnique (Emit®; Dade-Behring) on the Cobas Mira Plus analyzer. Hematologic side effects were defined according to the following criteria: white blood cells <3 × 10^9/μL, hemoglobin <70 g/L, and platelets <100 × 10^3/μL. For upper abdominal complaints, an esophagogastroduodenoscopy was performed. In the case of diarrhea, a bacteriological examination of stools was considered. The AUC for time 0–12 h (AUC[0–12]) was calculated at steady state, using the linear trapezoidal rule up to the last measured plasma concentration drawn 12 h after drug administration, by the following equation:

$$AUC_{[0–12]} = \sum_{i=1}^{n} \frac{c_i + (c_i + 1)}{2} \times [(t_i + 1) - t_i]$$

STATISTICAL METHODS

JMP 3.2 (SAS Institute Inc.) and Excel 97 (Microsoft Inc.) were used for statistics, calculations, and graphic interfaces. To compare MPA cimin, c30’, c60’, AUC[0–12], Tc cimin, the dose-normalized AUC, and MMF oral dose by body surface from patients with and without side effects, we used the two-sided t-test for comparison of means (two-sample t-test). For other comparisons, a paired t-test was used. Outliers were eliminated with the box plot if, in addition to statistical evidence, other reasonable evidence was present. The normality of 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 verified again. Even when normality could not be ob-

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4 The creatinine clearance was calculated according to the Cockroft-Gault formula (11). The dose-normalized AUC was calculated by dividing the AUC by the dose (mg/m²) and is expressed as (mg · h/L)/(mg/m²).
tained this way, we kept the results from parametric tests. When comparing these results with those obtained with the Wilcoxon signed-rank test, we could see no differences. The reported $P$ values are those of $\text{Prob} > |t|$. This is the $P$ value for observed significance of the two-tailed t-test. $\alpha$ was set at 0.05 for all analyses. ROC curves (12) were obtained using the MedCalc computer program (13).

### Results

**Clinical Results**

The median time for the first MPA PK assessment after transplantation was 8 days (range, 4–33 days). The median creatinine clearance at the first MPA PK assessment was 47.5 mL/min (range, 22.7–81 mL/min). Because the MMF oral dose was adjusted during the study according to the occurrence of side effects, the daily MMF dose (mg/day) at the end of the third month after transplantation was reduced to 250 mg twice a day in 10 patients (20%). In one case (2.0%), MMF was completely stopped. Thirty of 51 patients (59%) did not experience any side effects. In the remaining 21 patients, 33 episodes of side effects occurred during the study evaluation up to the third posttransplantation month. Side effect episodes included 2 thrombocytopenia, 8 leukopenia, 9 severe anemia, and 14 gastrointestinal adverse events (2 diarrhea with abdominal pain, 8 isolated diarrhea, and 4 esophagitis). The MMF oral dose by body surface (mg/m$^2$, twice a day) was 294.77 ± 36.06 mg/m$^2$ in the group of patients who experienced side effects and 278.02 ± 30.15 mg/m$^2$ in the group of patients who did not experience side effects ($P = 0.02$). At a fixed dose (500 mg twice a day), three patients presented with acute rejection (5.8%). Therapeutic monitoring obtained at the time of acute rejection revealed for these patients a MPA $c_{\text{min}}$ of 1.86, 1.76, and 3.83 mg/L, respectively, and a MPA AUC$_{(0-12)}$ of 37.7, 24.9, and 104.9 mg·h/L, respectively. The Tc $c_{\text{min}}$ was 5.4, 10.5, and 14.5 mg/L, respectively, for the three patients with acute rejection.

**PK Profile Analysis and PK/PD Relationship**

Of the total expected number of PK profiles, only 78 profiles were obtained and analyzed in patients treated with a fixed oral dose of MMF (500 mg twice a day). Forty-five PK profiles were obtained during the early posttransplantation period. The range, median, and mean ($\pm$ SD) of the MPA AUC$_{(0-12)}$ in these first PK profiles were 86.49 (maximum, 104.94; minimum, 18.45), 37.98, and 39.18 ± 15.78 mg·h/L, respectively. The range, median, and mean ($\pm$ SD) of the dose-normalized MPA AUC were 0.29 (maximum, 0.36; minimum, 0.07), 0.13, and 0.13 ± 0.06 (mg·h/L)/(mg/m$^2$), respectively. Fig. 1 illustrates the correlation between $c_{\text{min}}$, $c_{30}$, $c_{60}$, and AUC$_{(0-12)}$. Data used were those collected from PK profiles obtained during the entire study period. Linear correlation coefficients were significantly positive and were 0.68 ($P < 0.0001$) for $c_{\text{min}}$, 0.48 ($P < 0.0001$) for $c_{30}$, and 0.63 ($P < 0.0001$) for $c_{60}$. Interestingly, there was a significant positive correlation between MPA AUC$_{(0-12)}$ and MPA AUC$_{(0-2)}$ ($r = 0.68; P < 0.0001$).

Thirty-one PK profiles were obtained for 21 patients who experienced MPA toxicity. In these cases, the PK profiles were collected only if the patients were kept on a dose of 500 mg twice a day. The MMF oral dose change was made thereafter. Fifteen profiles were obtained in the early period after transplantation, 11 profiles between the early period and the third month, and 5 profiles at the end of the study period. Of the 47 PK profiles from the negative side effects group, 30 profiles were obtained in the early period after transplantation, 3 profiles between the early period and the third month, and 14 profiles at the end of the study period. The values for mean plasma $c_{\text{min}}$, $c_{30}$, $c_{60}$, AUC$_{(0-12)}$, and dose-normalized MPA AUC obtained for patients who underwent MMF toxicity or for
Table 1. Mean (± SD) MPA c_{min}, c_{30}, c_{60}, AUC_{(0–12)}, and dose-normalized MPA AUC according to side effects.

<table>
<thead>
<tr>
<th></th>
<th>With side effects (n = 31 samples)</th>
<th>Without side effects (n = 47 samples)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>c_{min}, mg/L</td>
<td>2.63 ± 1.58</td>
<td>1.75 ± 0.82</td>
<td>0.0167</td>
</tr>
<tr>
<td>c_{30}, mg/L</td>
<td>10.47 ± 6.27</td>
<td>7.66 ± 8.95</td>
<td>0.0091</td>
</tr>
<tr>
<td>c_{60}, mg/L</td>
<td>9.67 ± 5.42</td>
<td>8.53 ± 2.60</td>
<td>0.0002</td>
</tr>
<tr>
<td>AUC_{(0–12)}, mg·h/L</td>
<td>48.38 ± 18.50</td>
<td>36.04 ± 10.82</td>
<td>0.0006</td>
</tr>
<tr>
<td>Dose-normalized AUC, (mg·h/L)/(mg/m²)</td>
<td>0.16 ± 0.05</td>
<td>0.12 ± 0.04</td>
<td>0.0015</td>
</tr>
</tbody>
</table>

* Two-sample t-test.

patients free of side effects are shown in Table 1. All of these PK parameters were increased in the group of patients who experienced side effects when compared with the group of patients with an uneventful outcome: mean MPA c_{min} 2.63 ± 1.58 vs 1.75 ± 0.82 mg/L (P = 0.016); mean MPA c_{30} 10.47 ± 6.27 vs 7.66 ± 8.95 mg/L (P = 0.009); mean MPA c_{60} 9.67 ± 5.42 vs 8.53 ± 2.6 mg/L (P = 0.0002); mean MPA AUC_{(0–12)} 48.38 ± 18.5 vs 36.04 ± 10.82 mg·h/L (P = 0.0006); mean dose-normalized MPA AUC 0.16 ± 0.05 vs 0.12 ± 0.04 (mg·h/L)/(mg/m²) (P = 0.0015). The Tc c_{min} was similar in the two groups of patients: 12.25 ± 6.27 µg/L in the patients experiencing side effects vs 11.07 ± 3.7 µg/L in the patients with an uneventful outcome (P = 0.73).

ROC CURVE ANALYSIS AND TOXICITY

The ability of c_{min}, c_{max} [maximum MPA concentration during the first hour after oral dose (c_{30} or c_{60})], and AUC_{(0–12)} to discriminate MPA-related toxicity was evaluated by ROC curve analysis of the PK parameters derived from the 78 PK profiles. The areas under the ROC curves were, respectively, 0.67, 0.73, and 0.73 (Fig. 2). According to this analysis, the threshold of toxicity (MPA concentration leading to the best sensitivity and specificity combination) was 3 mg/L (sensitivity, 38.7%; specificity, 91.5%) for c_{min} 8.09 mg/L for maximum MPA concentration during the first hour (sensitivity, 77.8%; specificity, 67.4%), and 37.6 mg·h/L for MPA AUC_{(0–12)} (sensitivity, 83.3%; specificity, 59.6%). Comparison of the ROC curves did not demonstrate a statistically significant difference between c_{min} and AUC_{(0–12)} (P = 0.27), c_{max} and c_{min} (P = 0.58), and c_{max} and AUC_{(0–12)} (P = 0.8).

MPA PLASMA TRough CONCENTRATIONS AND AUC EVOLUTION OVER TIME

At the end of the study period, 17 PK profiles were obtained from 17 patients maintained at a fixed oral dose of MMF (500 mg twice a day). A comparison of MPA c_{min}, MPA AUC_{(0–12)}, and the dose-normalized MPA-AUC between the early posttransplantation period and 3 months is represented in Table 2. All of these parameters displayed a progressive increase during the first 3 months after transplantation. Nevertheless, they did not present statistically significant differences between these two pe-

riods: mean MPA c_{min} 1.66 ± 0.81 vs 2.23 ± 1.13 mg/L (P = 0.06); mean MPA AUC_{(0–12)} 34.85 ± 9.05 vs 41.84 ± 18.08 mg·h/L (P = 0.028); mean dose-normalized MPA AUC 0.12 ± 0.03 vs 0.13 ± 0.06 (mg·h/L)/(mg/m²) (P = 0.4). A statistically significant difference was observed for calculated creatinine clearance between these two periods: mean creatinine clearance, 46.58 ± 20.21 mL/min in the early posttransplantation period vs 62.84 ± 20.18 mL/min at 3 months (P = 0.012).

Discussion

Previous reports have shown the higher immunosuppressive efficacy of MMF in combination with calcineurin inhibitors in comparison with conventional therapy without MMF (14, 15). The rationale for this combination can be explained by the different modes of action of these drugs. In contrast to MMF, which blocks the de novo pathway of purine synthesis, calcineurin inhibitors (e.g., CsA and Tc) interfere with the early phase of lymphocyte response to antigen presentation by inhibiting cytokine synthesis by T lymphocytes. The European Multicenter Study (15) investigating the efficacy and safety of the combination of Tc and MMF therapy for the prevention of rejection following cadaveric kidney transplantation has already demonstrated encouraging results, confirmed by a subset population analysis reaching (Benelux experience) a full 2-year follow-up (16). One of the major conclusions of this study was that the acute rejection rates at 2 years provided by the combination of steroids and Tc, with either MMF (1 g/day) or with MMF (2 g/day), were similar (15% vs 12%). In a US prospective trial comparing Tc/prednisone with Tc/prednisone/MMF (2 g/day), although excellent patient and graft survival and a low incidence of acute rejection up to 6 months after grafting in the triple therapy group were observed (27% vs 44%), the average MMF dose at follow-up was 57% of the starting dose, which was reduced because of MMF-related side effects (17).

Our study, designed to investigate the PK basis of the combination of Tc with low-dose MMF and steroids, confirms early findings concerning the augmentation of MPA plasma concentrations when MMF is combined with Tc (8). According to our experience, the combination of Tc with MMF at a low dose (500 mg twice a day) provides PK values [c_{min} and AUC_{(0–12)}] equal to those obtained by the immunosuppressive regimen that includes CsA (Neoral) and MMF at 1 g twice a day (18). A recent report also reached the same conclusion in a pediatric population (19). To achieve an AUC of 50 mg·h/L, the oral MMF dose was 500 mg twice a day, without a calcineurin inhibitor, whereas it had to be reduced by 40% (300 mg/m² twice a day) when combined with Tc, and increased by 20% (60 mg/m² twice a day) when combined with CsA. A mechanism for the change in the pharmacokinetics of MMF by concomitant calcineurin inhibitors was investigated recently in an experimental model testing the inhibiting effect of Tc and CsA on the
enzyme UDP-glucuronyltransferase (20). This study provided evidence that Tc augments the bioavailability of MMF through the inhibition of MPA glucuronidation. Both CsA and Tc display an inhibitory behavior, but the calculated inhibition constants \( k_I \) of Tc and CsA for UDP-glucuronyltransferase were 27.3 ± 5.6 and 2518 ± 1473 mg/L, respectively. These in vitro findings explain why higher MPA concentrations occur in conjunction with lower plasma MPAG in Tc patients compared with CsA patients receiving the same MMF oral dose (8). However, it has already been documented from clinical studies that CsA affects the MPA \( c_{\text{min}} \), which is lower in patients receiving CsA than in patients not receiving CsA (21). In addition, other data have also shown a significant increase in MPA \( c_{\text{min}} \) after the withdrawal of CsA (22). A putative mechanism has been suggested, based on an experimental study, that CsA inhibits MPAG excretion into bile (23). As a result, the interaction between MMF and Tc or CsA is probably not attributable to one mechanism but is related to a possible inhibitory effect of Tc on MPA metabolism and to an inhibition of the enterohepatic recirculation of MPA by CsA.

The present study confirms the encouraging previous results on the efficacy of a Tc-MMF combined regimen even with a low MMF dose (500 mg twice a day). The acute rejection rate, up to the third month after transplantation, was 5.8%. However, 41% of the patients had MPA-related side effects during the study period, of either gastrointestinal (42%) or hematologic origin (58%). Dose reduction and discontinuation therapy occurred in 20% and 2.0% of cases, respectively. In the US study (17), 48% of patients had to discontinue MMF during the first 6 months because of similar side effects, but it is noteworthy that in this trial the MMF starting dose was 1 g twice a day. The high related side effect in our study, despite the low fixed MMF dose, may be explained by its design: the controlled variables were drug tolerance and efficacy at a fixed dose. The most frequent side effects related to MMF are leukopenia, diarrhea, and cytomegaloviral disease (24, 25). In our study, among the side effects were nine surprising episodes of anemia presumably related to

### Table 2. MPA plasma concentrations and creatinine clearance at early posttransplantation period and 3 months.

<table>
<thead>
<tr>
<th></th>
<th>Posttransplantation period(^a)</th>
<th>3 months(^a)</th>
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<tbody>
<tr>
<td></td>
<td>( n = 17 ) samples</td>
<td>( n = 17 ) samples</td>
</tr>
<tr>
<td>( c_{\text{min}}, \text{mg/L} )</td>
<td>1.66 ± 0.81(^b)</td>
<td>2.23 ± 1.13(^b)</td>
</tr>
<tr>
<td>( \text{AUC}_{(0-12)}, \text{mg} \cdot \text{h/L} )</td>
<td>34.85 ± 9.05(^c)</td>
<td>41.84 ± 18.08(^c)</td>
</tr>
<tr>
<td>Dose-normalized AUC, (( \text{mg} \cdot \text{h/L}/\text{mg/m}^2 ))</td>
<td>0.12 ± 0.03(^d)</td>
<td>0.13 ± 0.06(^d)</td>
</tr>
<tr>
<td>Creatinine clearance, ( \text{mL/min} )</td>
<td>46.58 ± 20.21(^e)</td>
<td>62.84 ± 20.18(^e)</td>
</tr>
</tbody>
</table>

\( ^a \) Mean ± SD.
\( ^b-e \) Paired t-test: \( ^b \) \( P = 0.06; \ ^c \) \( P = 0.28; \ ^d \) \( P = 0.4; \ ^e \) \( P = 0.012. \)
MMF therapy. A similar high incidence of anemia was reported in pediatric renal transplant recipients with presumed chronic rejection (26). In addition, a correlation between MPA \( c_{\text{min}} \) and a decrease in hemoglobin concentrations in stable renal transplant patients has been reported, suggesting that MPA has an effect on erythropoietic cells (27).

MPA measurement currently is achieved by two different analytical methods, HPLC (28) and the Emit assay (29). However, the Emit assay overestimates the concentration of MPA in plasma samples (30) because of a cross-reactivity with the M-2 metabolite (31, 32). Because the M-2 metabolite is known to inhibit, in vitro, the inosine monophosphate dehydrogenase activity (5), the Emit assay would better reflect the entire MMF immunosuppressive property. The positive bias varies from 7% to 35%, according to the organ transplanted (heart, liver, kidney) and concurrent immunosuppression (CsA or Tc) (31). For the kidney transplant population, the greatest bias is seen early after transplantation in patients receiving CsA (31). From a practical point of view, these variations should be taken into account, according to MPA measurement methods, when PK parameters are compared between studies.

TDM is the optimal way to achieve effective immunosuppression when drugs with interindividual variability are used (e.g., CsA, Tc, and recently MMF). Thus, optimizing immunosuppression therapy by maintaining a consistent target concentration is a primary goal in organ transplantation. For CsA as well as for Tc, the trough concentration is routinely used to tailor the individual dosage regimen for short- and long-term monitoring (33, 34), despite the fact that the AUC for both CsA and Tc may provide a more precise model for drug exposure after renal transplantation. There is increasing evidence that therapeutic MPA monitoring may help to improve outcome after renal transplantation for either efficacy or for side effects. The PK/PD relationship was clearly demonstrated in a previous study from our center of adult kidney transplant patients treated by the combination of CsA and MMF (1 g twice a day) (18). In this study, both MPA \( c_{\text{min}} \) and MPA AUC were higher in patients presenting with side effects than in those with uneventful outcomes. Moreover, at a fixed dose (2 g/day), the MPA concentration at 30 min (MPA \( c_{30} \)) was significantly associated with an increased risk for side effects. High MPA \( c_{30} \) concentrations would better explain the occurrence of side effects in patients with MPA AUCs within nontoxic limits. The Randomized Concentration Controlled Trial (RCCT) in an adult kidney transplant population also treated with CsA therapy has shown that variable target AUCs lead to a variable risk factor for acute rejection and adverse events (7). In addition, the RCCT study has shown that MMF safety is related more to the oral dose than to the MPA PK parameters (\( c_{\text{min}}, c_{\text{max}} \) and AUC). However, it should be mentioned that in the RCCT study there was a particular preponderance of gastrointestinal side effects, compared with other MMF-related adverse events, such as hematologic disorders. Additionally, the withdrawal rate from the study was significantly related to oral dose only for the gastrointestinal side effects and not for all adverse events. Recent data from a pediatric population (35) revealed a significant association between either the AUC\(_{0-12}\) for total MPA or MPA trough concentration and rejection outcome. In the latter work, the AUC for free MPA better discriminated patients with severe adverse events than the AUC\(_{0-12}\) for total MPA.

Currently, routine monitoring of MPA to individualize an oral regimen is still not widely adopted. The results of this study, designed to investigate the PK/PD relationship for MPA at a low MMF dose in combination with Tc, strongly support MPA monitoring as a guide for dosage adjustment to avoid inadequate immunosuppression. The present study demonstrated the relationship between plasma total MPA concentrations and toxicity. Both the trough concentration and AUC\(_{0-12}\) were significantly higher in the patient group experiencing side effects than in the group with an uneventful outcome. Interestingly, these results also show that the plasma total MPA concentrations obtained 30 and 60 min after oral dosing were significantly higher in the group that experienced side effects. Therefore, using ROC curve analyses, we tried to test the ability of \( c_{\text{min}} \), maximum total MPA concentration during the first hour after the oral MMF intake (\( c_{30} \) and \( c_{60} \), and AUC\(_{0-12}\) to discriminate between cases with or without side effects. The thresholds of toxicity were 3 mg/L, 7.4 mg/L, and 37.4 mg · h/L, respectively. A peak total MPA concentration >8 \( \mu \)g/L during the first hour, as well as the \( c_{\text{min}} \) and AUC\(_{0-12}\) appears to be an acceptable discriminator of adverse events in patients receiving MMF in combination with Tc. Another way to test this potential therapeutic strategy for routine monitoring of early MPA drug concentrations is demonstrated by the good correlation between AUC\(_{0-2}\) and AUC\(_{0-12}\).

In a TDM program, determination of the full MPA AUC\(_{0-12}\) in each patient may be impractical. To reduce blood loss, the need for longer hospital stays for blood sampling, and staff overtime, strategies have been developed to establish an abbreviated AUC. Proposed formulas are derived from a limited number of blood samples but are based on at least three MPA concentrations obtained up to 4 and 6 h after oral dose (36–38). Although there is a wide range of agreement between abbreviated AUCs derived from several models and full AUCs (39), such strategies are still unacceptable for ambulatory patients. From a practical point of view, this work supports the fact that when a fixed MMF dose is used in an effective immunosuppressive regimen, monitoring of toxicity based on either \( c_{\text{min}} \) or peak MPA concentration during the first hour (\( c_{30}, c_{60} \)) after oral dose could provide a simple and efficient tool to guide safe use of MMF to avoid an inappropriate reduction in or discontinuation of
therapy. Although not designed to establish a new AUC formula, this study shows that an AUC(0–2) based on four time concentrations ($c_{min}$, $c_{30}$, $c_{60}$, $c_{2h}$) may provide a practical way to estimate AUC(0–12).

At a fixed dose, MPA $c_{min}$/MPA AUC(0–12) and the dose-normalized MPA AUC displayed a progressive increase with time after transplantation concomitant with an increase in creatinine clearance. However, MPA concentration changes over time did not reach a statistically significant difference. This is likely because of the lack of sufficient statistical power in the small data set of $c_{min}$ and AUC. Several in vivo and in vitro studies in transplanted patients with several degrees of renal impairment have provided evidence of an alteration of MPA pharmacokinetics associated with renal function (40–43). The uremic state itself decreases MPA protein binding, which is also achieved by competition with the retained MPAG. The results of renal impairment are expressed by an increased AUC(0–12) for MPAG, MPA free fraction, and AUC(0–12) for free MPA. Recent data focusing on these PK changes, which occur over time after transplantation, have demonstrated that the temporary impairment of renal function early after transplantation lowers the MPA AUC by increasing the MPA oral clearance (44). Improving renal function up to the third month after transplantation, which is revealed by an increase in creatinine clearance, leads to a normalization of MPA albumin binding and thus to the observed change of MPA pharmacokinetics. This is the reason that MPA pharmacokinetics were first determined in this study when the creatinine clearance had reached 40 mL/min, in the majority of cases, to minimize alterations in MPA pharmacokinetics by renal function. Other factors influencing MPA metabolism may include changes in enterohepatic recirculation and induction of hepatic glucuronyl transferase activity by glucocorticoids (45), which usually are administered in high doses early after transplantation and tapered thereafter. Nevertheless, from a practical point of view, monitoring of free MPA would be indicated in clinical conditions that alter protein binding and total MPA concentrations.

In conclusion, monitoring of plasma MPA after kidney transplantation in patients receiving an immunosuppressive regimen combining Tc and MMF demonstrates the relationship between plasma MPA and toxicity. Our results suggest that a PK strategy would optimize MMF therapy in kidney transplantation and also support the need for MPA TDM. In routine clinical practice, the MPA $c_{min}$ and peak concentration during the first hour after oral dose ($c_{30}$, $c_{60}$) show a significant correlation with the occurrence of adverse events as indicated by the AUC(0–12). An MMF oral dosing strategy related to the PK parameter thresholds as defined in this work could be assessed in a prospective way to confirm that TDM may improve patient outcome.

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References


