Why and How to Perform Therapeutic Drug Monitoring for Mycophenolate Mofetil

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Abstract

The introduction of the immunosuppressive agent mycophenolate mofetil as a standard-dose drug has resulted in a reduced risk of rejection after renal transplantation and improved graft survival compared to azathioprine. The favorable balance between efficacy and safety has made mycophenolate mofetil a cornerstone immunosuppressive drug, and the vast majority of newly transplanted patients are now started on mycophenolate mofetil therapy.

Despite the obvious success of mycophenolate mofetil as a standard-dose drug, there is reason to believe that the "one size fits all" approach is not optimal. Recent studies have shown that the pharmacokinetics of mycophenolic acid are influenced by patient characteristics such as gender, time after transplantation, serum albumin concentration, renal function, co-medication, and pharmacogenetic factors. As a result, with standard-dose therapy there is wide between-patient variability in exposure of mycophenolic acid. This variability is of clinical relevance because it has repeatedly been shown that exposure to the active metabolite mycophenolic acid is correlated with the risk of developing acute rejection. Especially with the increasing popularity of immunosuppressive regimens in which the concomitant immunosuppression is reduced or eliminated, ensuring an appropriate level of immunosuppression afforded by mycophenolic acid is of utmost importance.

By introducing therapeutic drug monitoring, mycophenolic acid exposure can be targeted to the widely accepted therapeutic window (mycophenolic acid AUC_{0-12} between 30 and 60 mg·h/l). Three prospective randomized studies comparing concentration-controlled mycophenolate mofetil therapy to a fixed-dose regimen will further clarify the role of therapeutic drug monitoring in increasing the therapeutic potential of mycophenolate mofetil. (Trends in Transplant 2007;1:24-34)

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Key words

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Introduction

Mycophenolate mofetil (MMF, Cellcept®, Roche) was introduced in 1995 for the prevention of acute rejection in renal-allograft recipients[1]. The active metabolite mycophenolic acid (MPA) is a selective, reversible, noncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPDH). This enzyme is an important step in the *de novo* synthesis of guanine nucleotides. MPA inhibits T- and B-lymphocyte proliferation by inhibition of IMPDH activity, which ultimately results in its immunosuppressive effect.

A pooled efficacy analysis of the registration trials found a significant decrease in the rate of rejection in renal-transplant recipients, from 40.8% in placebo or azathioprine treatment to 19.8 and 16.5% for the groups treated with MMF 2 g and 3 g, respectively[2]. The favorable balance between efficacy and safety has made MMF a cornerstone immunosuppressive drug, and the vast majority of newly transplanted patients are now started on MMF therapy[3]. Its lack of nephrotoxicity has made MMF also very popular in reduced-toxicity regimens, involving the minimization or withdrawal of calcineurin inhibitors or corticosteroids[4].

Although MMF was initially introduced as a standard-dose drug at a fixed-dose regime of 1 g orally twice daily, at present therapeutic drug monitoring (TDM) is a frequently discussed topic that may further improve MMF therapy. In this review the determinants of variability in MPA exposure are shown, and the rationale for TDM is discussed.

We would like to emphasize that with the increasing use of MMF in other types of solid-organ transplantation, stem-cell transplantation, and autoimmune diseases, the same issues already do or will play a role. The need for TDM may also apply to these indications. Furthermore, we want to stress that because of different pharmacokinetics, the data in this manuscript only relate to the MMF formulation and not to the enteric-coated mycophenolic sodium or other (generic) MPA formulations.

Why is there a need for therapeutic drug monitoring?

For a drug to be considered a suitable candidate for TDM a number of criteria need to be met. These criteria include a clear relationship between drug concentration and effect, a small therapeutic index, and considerable between-patient pharmacokinetic variability[5]. Several papers with recommendations on when and how to perform TDM for MMF have been published[6,7]. However, in view of the cost and effort involved in performing TDM, more evidence for the validity of a dose-individualization approach is needed as a basis for decision making in healthcare policy. Randomized, multicenter, prospective trials have been started to supply the highest grade of evidence as well as a quantification of the impact of TDM. In these trials, the added value of TDM of MPA is investigated by comparing fixed-dose MMF treatment with concentration-controlled treatment in *de novo* kidney transplant recipients. If such studies show an improvement in clinical outcome for patients in whom TDM is performed, compared to a control group on standard-dose therapy, then this will give further support to the implementation of routine pharmacokinetic monitoring and dose individualization. Remarkably, for cyclosporine and tacrolimus, drugs for which routine TDM is performed worldwide, there have been no randomized studies to examine the potential benefit of TDM. Nevertheless, there is little controversy among clinicians that measuring cyclosporine and tacrolimus in blood is a useful adjunct to their optimal administration. In fact, the Fixed Dose versus Concentration Controlled (FDCC) study, initiated in 2003 and investigating the value of monitoring MPA concentrations, is the first randomized study for immunosuppressive drug monitoring[8].

The rationale behind studies investigating the potential of TDM for MMF is twofold. First, with standard-dose therapy there is wide between-patient variability in exposure of MPA. And second, this variability is of clinical relevance as exposure to the active metabolite MPA is correlated with the risk of allograft rejection.

MPA exposure and efficacy

Several clinical studies with MMF have shown that while no correlation was observed between MMF dose and allograft rejection, the pharmacokinetic parameters of MPA did show a relationship with efficacy[9-11]. In these studies, it was shown that patients developing biopsy proven acute rejection had lower drug exposure (either MPA AUC_{0-12} and/or predose levels) compared to non-rejecting patients[12]. The MPA AUC_{0-12} has a better correlation with the risk of rejection than predose levels[10,13,14].

A hallmark study for the interest in measuring MPA levels was the Randomized Concentration-Controlled Trial (RCCT). In this study, 150 renal-transplant patients treated with MMF, cyclosporin A (CsA) and prednisone for six months were randomized to three AUC target groups: low (16.1 mg·h/l), intermediate (30.3 mg·h/l) and high (60.6 mg·h/l). The patients in the low target MPA AUC group had the highest risk of biopsy proven acute rejection. Using logistic regression analysis, a statistically significant relationship was found between the incidence of biopsy proven acute rejection and MPA AUC (p < 0.001) and MPA C_0 (p = 0.01), with the AUC showing the best correlation[10]. Similar correlations between pharmacokinetic parameters and outcome have been reported by the German study group on MMF therapy in pediatric renal-transplant patients[9]. They found an increased risk for acute rejection in the first month posttransplantation in children with MPA AUC_{0-12} values < 33.8 mg·h/l (p = 0.005) or predose levels < 1.2 mg·h/l (p < 0.001)[9]. Also, Kiberd, et al. showed the importance of adequate MPA exposure as early as day three in an observational study with 94 CsA and MMF treated renal-transplant recipients. In these patients, the MPA AUC_{0-12} (estimated with limited sampling) at day three was strongly associated with increased risk of acute rejection[11]. Remarkably, in this study the C-2 cyclosporine concentration was not significantly related to acute rejection. Also remarkable is that in this study, in the majority of patients, induction therapy with basiliximab was used. One

would think that because of the induction therapy, early drug exposure to MPA would not be so critical. In this study, however, the day three exposure to MPA was an important predictor of acute rejection.

Influence of co-therapy with cyclosporine or tacrolimus

In the three registration studies and in the abovementioned studies, all included patients were on CsA therapy co-therapy[9-11]. Currently, an increasing proportion of patients are being co-treated with tacrolimus[3]. The MPA pharmacokinetics are different if MMF is used in combination with tacrolimus or CsA. Increased MPA clearance and decreased-dose normalized concentrations of MPA are found in CsA co-treated patients compared to tacrolimus co-therapy[15]. Although initially it was unclear if it was CsA that caused a decrease in MPA exposure, or tacrolimus that increased levels, the evidence now points towards a role for CsA. It was also shown that discontinuation of CsA in the immunosuppressive therapy leads to increased MPA predose levels[16], suggesting that the differences depend on an effect of CsA on the pharmacokinetics of MPA. The "Creeping Creatinine-study", where CsA was discontinued, showed an increased incidence of anemia, despite improved allograft function, most likely due to a rise in MPA concentrations[17,18].

The assumed mechanism for the interaction between CsA and MPA is inhibition of the enterohepatic recirculation of MPA glucuronide (MPAG) due to an effect on the multidrug resistance-associated protein 2 (MRP2) enzyme[19]. The MRP2 is responsible for the excretion of MPAG in bile[20,21]. Cyclosporin A inhibits the excretion of MPAG, which also explains the elevated MPAG plasma concentrations. Cremers, et al. also found evidence for the inhibitory effect of CsA on the enterohepatic recirculation of MPA in a study where they showed that total MPAG clearance was lower in CsA co-treated patients than in tacrolimus co-treated patients, despite a simi-

lar renal function in both groups[22]. It can not be completely ruled out that tacrolimus also has an effect on MPA pharmacokinetics, but if there is one it is much smaller than the influence of CsA. Arguments for a role of tacrolimus can be found in studies where a decreased MPA clearance was found if MMF was combined with tacrolimus compared to no calcineurin inhibitor[23]. Possibly, tacrolimus would have this effect through inhibition of UDP-glucuronosyltransferase (UGT), the enzyme responsible for the formation of MPA to the inactive metabolite MPAG[24].

Not only is the MPA pharmacokinetics different if MMF is used in combination with tacrolimus or CsA, but it also seems that the relationship between MPA concentrations and clinical outcome is less convincing for tacrolimus. In a Belgian study, 100 renal-transplant patients treated with MMF and tacrolimus were followed for 12 months[25]. Simultaneous tacrolimus and MPA AUC measurements were performed at five time-points within the first year posttransplantation. Despite the intensive pharmacokinetic monitoring and the relatively large sample size, in this study there was no more than a trend towards a higher incidence of acute rejection in recipients who did not reach both a target tacrolimus AUC_{0-12} of 150 mg·h/l and an MPA AUC_{0-12} of 45 mg·h/l (p = 0.07)[25]. Also, MPA exposure was not related to the occurrence of infectious complications, whereas this was related to tacrolimus exposure[25]. Anemia and leukopenia were related to MPA exposure.

MPA exposure and toxicity

The most frequently reported side effects in MMF therapy are gastrointestinal symptoms, hematologic disorders, and infections[26]. The relationship between MPA exposure and adverse events is, however, not as well established as the correlation with the risk of acute rejection. In part this is due to the lower incidence of some of these adverse events, but also it can be difficult to distinguish MMF-related adverse events from adverse events caused by other factors, or caused by concurrently used (immunosuppressive) drugs. Some large studies were not able to find a relationship between MPA pharmacokinetic parameters and toxicity[11,14]. In the RCCT study (n = 150), no significant correlation was seen between adverse events and MPA C_0, MPA C_{max} or MPA AUC_{0-12}, whereas the MMF dose was significantly related to the occurrence of adverse events, which were mostly of gastrointestinal origin in this trial[14]. This may have been caused by the study design and by the method of statistical analysis. In the RCCT study, the incidence of adverse events was correlated with the mean of the AUC values available for each patient. Due to the double-blind study design of the RCCT trial, patients suffering from adverse events that might be due to MMF therapy had to be withdrawn from the study. As a result, the total follow-up time for these patients in the study was relatively short. Patients without adverse events typically had full six-month follow-up and completed all assessments of MPA exposure. Because MPA exposure shows a gradual increase over time, patients with adverse events (who discontinued earlier and had shorter follow-up time) had MPA exposure that was rather low, compared to the patients without adverse events that reached the later pharmacokinetics sampling time-points and therefore reached higher MPA exposure. This may have obscured the relationship between high MPA concentrations and side effects.

Analyses by Kiberd, et al. (n = 94) for predictors of toxicity were also negative for MPA exposure (AUC_{0-12}, C_0 and C_2). Toxicity was defined here as the need to reduce or discontinue the dose of MMF for clinical symptoms or for abnormal laboratory values[11]. Nevertheless, other smaller studies did find a correlation between MPA exposure and adverse events. A significant relationship between adverse events, especially leukopenia and anemia, and MPA exposure (AUC_{0-12}, C_0 and C_{max}) was seen in renal-transplant recipients co-treated with CsA or tacrolimus[25,27,28]. In none of these trials was the non protein-bound (free) concentration of MPA determined. In studies where free MPA was monitored, pa-

tients who experienced infections or hematologic events, including leukopenia, had a significantly higher free-MPA AUC[9,29]. Surprisingly, the relationship between free-MPA concentrations and efficacy has been generally poor and certainly not better than for total MPA exposure[30]. For toxicity (especially infections and hematologic toxicity), unbound MPA concentrations may be more relevant.

Between-patient variability

Based on the abovementioned clinical studies, a therapeutic window for MPA AUC_{0-12} of 30-60 mg·h/l has been proposed[31]. This therapeutic window for MPA AUC_{0-12} is comparable with predose levels in the target range of 1-3.5 mg/l, or 1.7-4.0 mg/l when MMF is combined with tacrolimus[32]. In patients on standard-dose MMF therapy, MPA pharmacokinetics exhibit large inter- and intrapatient variability in both AUC_{0-12} and predose levels[1]. The MPA AUC_{0-12} in renal-transplant recipients after administration of 1 g MMF ranges between approximately 10 and 100 mg·h/l[30]. The MPA clearance following other kinds of transplantation (liver, heart, and bone marrow) shows a similar variability[32-34]. Several factors such as co-medication and time after transplantation influence the MPA exposure. Intrapatient variability in MPA exposure is relatively low compared to between-patient variability[33]. Pharmacokinetic monitoring is expected to increase the effect of MMF treatment because it would increase the amount of patients on target due to the large between-patient variability and small intrapatient variability.

Pharmacodynamic monitoring

Theoretically, it is more logical to monitor a drug by measuring its biologic effect than merely its concentration in blood. Patients may differ regarding their susceptibility for a particular compound, which will be missed by focusing on pharmacokinetics only. However, in clinical practice, pharmacodynamic monitoring of immunosuppressive drugs is still somewhat unexplored. Detailed knowledge of the exact mechanism of action allows for the development of assays that can be used to monitor the pharmacodynamic effect.

The mechanism of action of MPA is the inhibition of IMPDH. For lymphocytes, this enzyme forms a crucial step in the *de novo* synthesis of guanine nucleotides. Similar to MPA pharmacokinetics, the activity of IMPDH displays high between-patient variability. This variability was observed in both healthy volunteers and pre- and posttransplantation in renal-transplant recipients[35-37]. Several studies have shown an inverse relationship between plasma MPA concentration and IMPDH activity throughout the MMF dose interval[35,38,39]. Investigators in Berlin found associations between low pretransplant IMPDH activity and the need for dose reduction due to adverse events (p < 0.004) and between high pretransplant IMPDH activity and rejection (p < 0.01)[36]. This means that patients with low pretransplant IMPDH activity need less MMF to get the same immunosuppressive effect. These findings suggest that pharmacodynamic monitoring of IMPDH activity may be suitable to individualize MMF therapy[40]. Whether the observed between-patient variability in IMPDH activity is linked to polymorphisms in the IMPDH gene remains to be determined.

Determinants of MPA exposure

Gender

The effect of gender on MPA pharmacokinetics has been studied by numerous authors, and the results of these studies are not equivocal. Morissette, et al. found gender-related differences in the MPAG/MPA ratio. The average MPAG/MPA ratio was significantly increased in men compared to women[41]. Borrows, et al. found increased MPA trough levels in female renal-transplant recipients compared to males (p = 0.002)[42]. A competitive inhibition of UGT enzymes by estrogen may explain the gender differences[41].

The relationship between patient factors and pharmacokinetic parameters has also been studied by developing a population pharmacokinetic model for MPA following oral administration of MMF. In the final model, it appears that males have an 11% higher MPA clearance than females[43]. Other studies found no effect of gender on the pharmacokinetics of MPA[44-46]. In one study, the mean AUC in females was higher than in males, but this difference was not significant (ratio female/male = 1.094; 90% CI: 0.975-1.228)[44]. In a population pharmacokinetic meta-analysis containing 13,346 MPA concentration-time data points from 468 renal-transplant patients, also no significant relationship was found between gender and MPA exposure[45]. Overall, the studies give conflicting results. Some small studies suggest that MPA metabolism is reduced in women, but a meta-analysis found no correlation between gender and MPA pharmacokinetics.

Race

A recent study in Chinese patients showed that the between-patient variability in MPA pharmacokinetics is similar to Caucasian patients[47]. Chinese patients, however, seem to have a lower MPA clearance and higher MPA exposure with equivalent dosing. As the investigators also observed a relatively high incidence of adverse events related to conventional-dose MMF therapy, they conclude that plasma MPA monitoring in their patients may assist in identifying patients with supra-therapeutic drug exposure.

African American kidney transplant patients have been recognized to be at higher risk for early acute-rejection episodes[48]. African American renal-transplant recipients have significantly less benefit of MMF treatment, considering risk of acute rejection compared to Caucasians[49]. The benefit of MMF compared to azathioprine on long-term outcomes is equivalent for both ethnicities[50]. To produce comparable benefit/risk ratios in African American renal-allograft recipients as in non-African American renal-transplant

patients, higher MMF doses may be required. In African American renal-transplant patients, a higher MMF dose is needed to achieve a significant benefit in acute rejection compared to Caucasians (1.5 and 1.0 g 2/d, respectively)[48]. This difference in clinical outcome between African American and Caucasian patients can not be explained by a difference in MPA exposure, as no significant differences in the pharmacokinetics of MPA were found between African American and Caucasian stable renal-transplant recipients. The exposure to both MPA and MPAG (defined by AUC_{0-12}, C_{max} or C_0) was comparable between the two ethnic populations[44,51]. The variability in MPA trough levels was also found to be unaffected by ethnicity in comparison with other races (Caucasian, Indo-Asian, Afro-Caribbean)[42]. These results indicate that the racial differences in renal-graft survival are not caused by pharmacokinetic differences. The explanation for the requirement of higher MMF doses in African American transplant patients must be sought elsewhere. The increased risk of rejection in African American renal-transplant recipients is probably caused by heightened immune responsiveness[51,52]. This stresses the importance of adequate immunosuppressive drug exposure with target differentiation depending on race to achieve better outcome[51,52].

Bodyweight

MMF is not dosed on a per-kilogram basis. A population pharmacokinetic analysis of Le Guellec, et al. (n = 60) found that bodyweight was positively correlated with oral MMF clearance. Bodyweight in the pharmacokinetic model reduced the unexplained variability in clearance from 34.8 to 28.2%. The magnitude of this reduction in variability is not sufficiently strong to recommend dosing on a per-kilogram basis[53]. In the study of Staatz, et al. (n = 117), a trend towards increased MPA clearance with higher bodyweight was found. Inclusion of patient weight into the model resulted in 1.3% absolute reduction in between-patient variability[54]. Other large trials of van Hest, et al. (n = 468), Kuypers, et al. (n = 100),

and Borrows, et al. (n = 117) did not show a correlation between bodyweight and MPA pharmacokinetics[42,45,46]. These results suggest that dosing MMF based on bodyweight will not improve MPA exposure.

Diabetes

We analyzed the influence of diabetes on MPA pharmacokinetics in a retrospective analysis of the RCCT data set. No significant differences in MPA exposure (AUC_{0-12}, C_l and C_{max}) between diabetic (n = 7) and nondiabetic (n = 129) kidney transplant patients were found. However, in diabetic recipients, T_{max} of MPA was significantly increased on day 11 after transplantation (1.59 hours for diabetic versus 0.67 hours for nondiabetic patients; p = 0.04)[55]. A subsequent population pharmacokinetic meta-analysis of 468 renal-transplant patients confirmed an increased T_{max} (p = 0.045) in patients with diabetes[45]. The delayed T_{max} is likely to be caused by slower absorption as a consequence of gastroparesis[45,55]. This did not influence overall MPA exposure over the 12-hour dosing interval. Other studies also found no difference in total MPA exposure between diabetics and nondiabetics after renal transplantation[44,54].

Protein binding of MPA

MPA is extensively bound to serum albumin under normal conditions (binding ± 97%). Consistent with the behavior of numerous other drugs, the free fraction is thought to be responsible for the immunosuppressive effect of MPA[1]. *In vitro* studies have demonstrated that the free fraction inhibits the target enzyme, IMPDH[56]. *In vivo*, increased exposure to free MPA causes an elevated risk of certain MMF-related side effects[9,29] Clearance of MPA is proportionally increased with increasing free fraction[29]. A decrease in free-MPA levels leads to a reduction of MPA clearance and increase in total MPA levels[6,57]. The influence of albumin concentration on MPA

clearance and exposure was confirmed in two population pharmacokinetic models[43,54].

The MPA free fraction depends on the serum albumin concentration and renal function of the patient. An increased albumin concentration is correlated with a decrease in free fraction and free MPA AUC_{0-12}[29,56,57]. Atcheson, et al. reported 70% higher MPA free fraction in patients with albumin concentrations < 32 g/l[29,58]. Early after transplantation, and in particular in liver-transplant patients, such albumin concentrations are not infrequently found.

Renal function

Increased serum creatinine and decreased glomerular filtration rate (GFR) are also associated with decreased MPA predose levels[42]. Uremic serum results in a decrease in MPA albumin binding and in an increase of the metabolite MPAG, which has been shown *in vitro* to compete with MPA for binding sites on albumin[51]. As one would expect, renal dysfunction leads to decreased MPA concentrations, increased MPAG concentrations, and increased free-MPA fractions[51,59]. Case reports on patients with severe renal insufficiency and markedly increased free-MPA levels associated with toxicity have been published[60]. In a pharmacokinetic population model, reduced creatinine clearance correlated significantly with increased MPA clearance[43]. The creatinine clearance accounts for 19% of the intrapatient variability of MPA clearance[61].

In clinical practice, and especially in kidney transplantation from deceased donors, delayed graft function affects about 25% of all patients. The patients suffering from delayed graft function have a prolonged period of poor renal function, remain dialysis-dependent for some days to weeks, and meanwhile are exposed to reduced MPA concentrations. This is unfortunate as we know that patients with delayed graft function are at an increased risk of acute rejection[59].

Pharmacogenetic variation and pharmacokinetics

If we focus on the influence of gene polymorphisms on pharmacokinetic variability of MPA, the UGT and MRP2 transporter are most relevant.

The most important UGT isoforms for the glucuronidation to MPAG and acyl MPAG are UGT1A9 and UGT2B7, respectively[62,63]. It appears that UGT1A9 is the most important UGT isoform for the glucuronidation of MPA, accounting for more than 50% of MPAG production in liver, kidney, and intestinal mucosa. In addition, MPAG is formed by UGT1A7, UGT1A8, and UGT1A10, which are expressed in the kidney and gastrointestinal tract. The UGT2B7 is responsible for the formation of acyl MPAG[63-66].

In a recent study, Girard, et al.[67] found a 17-fold variation in the amount of UGT1A9 protein in adult human livers[67]. The MPA glucuronidation activity in hepatic microsomes differed more than 9.5-fold and was significantly correlated with UGT1A9 protein levels[67].

Evidence for a genetic basis of the variable UGT expression was provided recently with the identification of several single nucleotide polymorphisms (SNP) in the UGT1A1, UGT1A7, and UGT2B7 genes. Some of these SNP result in the complete or partial loss of glucuronidation activity[68]. In addition, SNP have been discovered in the coding and promoter region of the UGT-1A9 gene, which is considered to be the UGT isozyme most important for MPA glucuronidation[67,69]. Of all UGT1A9 promoter SNP investigated, the –2152C>T and –275T>A SNP were found to have the strongest association with hepatic UGT1A9 protein content[67]. Carriers of these closely linked SNP had roughly two-fold higher UGT1A9 protein levels compared with carriers of the wild-type promoter and with non-carriers of the –2152C>T/–275T>A SNP. Importantly, *in vitro* MPA glucuronidation activity was 2.1-fold higher in –2152C>T/–275T>A carriers[67].

Kuypers, et al. reported that the –2152C>T and –275T>A SNP in UGT1A9 are associated with significantly lower MPA exposure in the early phase after renal transplantation[70]. However, the association between genotype and MPA exposure could only be demonstrated for patients treated with 2 g/d, and not for patients on 1 g/d. Also, a significant relationship was only found on day seven, and not at the other three time-points after transplantation. Given the overlap in MPA exposure in carriers and non-carriers, it is questionable if these findings are clinically relevant and truly offer a means for a personalization of MMF treatment. The less frequent UGT1A9*3 SNP, present in less than 5% of the Caucasian population, was associated with a higher MPA exposure, which is in agreement with the previously described reduction of *in vitro* enzymatic activity[67,70]. Another interesting observation was the finding that the MMF-related gastrointestinal side effects occurred numerically (but not statistically significantly) less frequently in carriers of the UGT promoter SNP.

Mutation of the UGT2B7 gene is associated with a significantly higher acyl MPAG/MPA ratio (respectively 2- and 2.6-fold higher ratios in heterozygous and homozygous mutated patients; $p < 0.05$) due to an increased production of acyl MPAG[71].

MRP2 is responsible for the biliary excretion of MPAG. Most likely, this same transporter is responsible for the active secretion of MPAG into urine. Cyclosporin A reduces the enterohepatic recirculation of MPA through inhibition of MRP2[19,20]. We determined the impact of MRP2 gene polymorphism on MPA exposure in renal-transplant patients. Heterozygosity for the *C-3972T* SNP was found in 117/259 and homozygosity in 28/259 of the patients. Carriers of the *C-3972T* polymorphism in the MRP2 gene had little but significantly decreased MPA AUC_{0-12} (59.0 vs. 64.7 mg·h/l; $p = 0.045$) when tacrolimus was coadministered[72].

Naesens, et al. also studied MRP2 polymorphisms (*C-24T* and *C-3972T*) and MPA phar-

macokinetics[73]. No differences in MPA exposure were noted between carriers and non-carriers of the *C-24T* SNP on day seven, but at later time-points dose-normalized MPA AUC was consistently higher in carriers of the *C-24T* SNP.

Pharmacogenetic variation and pharmacodynamics

The IMPDH enzyme consists in two isoforms, type I and type II. Type I is constitutively expressed, while type II is expressed upon immune activation. The MPA has a fivefold higher inhibitory affinity for IMPDH-II than IMPDH-I[74]. Therefore, pharmacogenetic analysis of IMPDH-II may contribute more to individualization of MMF therapy[75]. The SNP of both isoforms have been identified, but no associations between these polymorphisms and the incidence of acute rejection were detected[76-78]. However, Grinyo, et al. found a significant increased acute rejection in carriers of the *T-3757C* polymorphism of IMPDH-II (OR: 2.99; CI: 1.27-6.99; p = 0.012)[79].

Therapeutic drug monitoring of MPA

The large between-patient variability and the established concentration-effect relationship suggest that TDM may be used as a tool to optimize MMF treatment in renal-transplant recipients. Before starting a prospective randomized study, we performed a computer simulation to study the feasibility of TDM for MMF[80]. Such trial simulations are increasingly important in selecting a trial design that will generate the maximum amount of information on the drug under investigation. First, by using a nonlinear mixed-effects model for MPA, Bayesian estimates for MPA oral clearance in the first six months after transplantation were provided. Subsequently, using these estimates, MPA AUC values were calculated for a cohort of patients, and exposure to MPA was compared for a situation of standard dosing versus concentration-controlled dosing. We showed that in the concentration-controlled group the tar-

get concentrations were reached more quickly and in a higher proportion of patients, and that between-patient variability was reduced. Only 13% of the patients receiving MMF 1 g 2/d had an MPA AUC between 30 and 60 mg·h/l one week after transplantation, which increased in three months to 67% of the patients[80]. We also found that in this cyclosporine-treated population, to reach target MPA exposure in the first weeks after transplantation, higher doses of MMF are necessary than currently recommended.

There is increasing recognition of the potential that the variability in both pharmacokinetics and pharmacodynamics may lead to new strategies in individualizing MMF treatment. Fixed-dose therapy may not be the optimal dosing strategy. Ten years after introduction of MMF into the clinical arena, we should be open for new data that put our current dosing regimens under discussion. Meanwhile, data from three clinical trials comparing the efficacy and cost-effectiveness of fixed-dose versus concentration-controlled MMF dosing will be published in 2007 (the Fixed Dose versus Concentration Controlled study, the Apomygre study, and the OptiCept study[81-83]). In all three trials a pharmacogenetic substudy has been added. The MPAG and free MPA exposure will also be measured in a subset of patients. These trials will be able to answer the question whether the current "one MMF dose fits all" should be replaced by a dosing scheme based on individual MPA plasma concentrations.

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