The Journal of Applied Pharmacology, 13, 65-77(2005)

Clinical Pharmacology of Mycophenolic Acid as Immunosuppressant in Organ Transplantation

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(Received Novemer 23, 2004; Accepted January 4, 2005)

Abstract – Present article reviews about clinical pharmacology of mycophenolic acid (MPA), the active form of mycophenolate mofetil (MMF), as widely used component of immunosuppressive regimens in the organ transplantation field. MMF, used alone or concomitantly with cyclosporine or tacrolimus, has approved in reducing the incidence of acute rejection and has gained widespread use in solid organ such as kidney, heart and liver transplantation. The application of MPA and development of MMF has shown a considerable impact on immunosuppressive therapy for organ transplantation as a new immunosuppressive agent with a different mechanism of action from other drugs after early 1990s. In particular aspect, use of MMF, a morpholinoethyl ester of MPA, represented a significant advance in the prevention of organ allograft rejection as well as allograft and patient survival. In considering MMF clinical data, it is important to note that there is a strong correlation between high MPA area under curve (AUC) values and a low probability of acute allograft rejection. Individual trials have shown that MMF is generally well tolerated and revealed that MMF decreased the relative risk of developing chronic allograft rejection compared with azathioprine. Recent clinical investigations suggested that improved effectiveness and tolerability will results from the incorporation of MPA therapeutic drug monitoring into routine clinical practice, providing effective MMF dose individualization in renal and heart transplant patients. Therefore, MMF has a selective immunosuppressive effect with minimal toxicity and has shown to be more effective that other agents as next step of immunosuppressive agents and regimens that deliver effective graft protection and immunosuppression along with a more favorable side effect.

Keywords □ Clinical pharmacology, immunosuppressive agents, mycophenolic acid (MPA)

INTRODUCTION

Mycophenolate mofetil (MMF), a prodrug immunosuppressant, has been approved for prevention of acute rejection in the heart and kidney transplants recipients (Sollinger, 1995; Tricontinental trial, 1996; Kobashigawa *et al.*, 1998; Mathew, 1998). MMF having a half-life of less than 2 minutes when given intravenously is rapidly converted to the active mycophenolic acid (MPA) that is metabolized to the MPA glucuronide (MPAG) by UDP-glucuronosyl-transferase, which is mostly eliminated in the urine, with small amounts in the feces (Fulton and Markham, 1996). Plasma MPA-C_{max} occurs about 2 hrs after oral administration and it is further metabolized to MPAG but convert to MPA by gut flora, and reabsorption of the MPA into blood stream, so called enterohepatic recylcling, resulting in a secondary peak level 6-8 hrs later (Shaw *et al.*, 1998; Lee *et al.*, 1990). MPAG is thought to have no efficacy but may be related with some side effects (Lipsky, 1996; Bullingham *et al.*, 1998; Shipkova *et al.*, 1999).

The another metabolite of MPA, the acyl glucuronide that have some efficacies such as promotion of interleukin (IL)-6 and TNF-α release related with some toxic effect has been described (Schutz *et al.*, 1999; Wieland *et al.*, 2000). The

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effects of MMF on rat lymphocytes have shown a significant relationship between MPA pharmacokinetics and inhibition of not only mitogen-stimulated lymphocyte proliferation, but also lymphocyte expression of cell surface cytokine receptors and these mechanisms of action may contribute to the its therapeutic efficacy in vivo (Gummert *et al.*, 1999; Ransom, 1995).

In inbred *Lewis* rat heart transplants model, MMF showed the equipotent suppressive effects on both lymphocyte proliferation and activation and its effects were highly correlated with plasma MPA levels. A statistically significant relationship between MPA-AUC, whole blood pharmacodynamic assays of lymphocyte proliferation, and median graft rejection histology scores provide support for a relationship between plasma MPA level and the risk for acute rejection in organ transplant patients.

Prostaglandin (PG)E_2 plays an important role in cellular immune functions that inhibits T-cell mitogenesis, IL-2 production, and IL-2 receptor expression. MMF strongly diminished the PGE_2 level in the cell culture supernatant in a concentration-dependent manner (Demeure *et al.*, 1997). MMF strongly down-regulates the release of PGE_2 by activated endothelial cell (Blaheta *et al.*, 2000) and an increased PGE_2 level correlates with an enhanced allograft survival after liver allograft in rat model (Arai *et al.*, 1999; Perez *et al.*, 1998). Therefore, MMF effect on PGE_2 production may be play an important role in immunosuppressant therapy in organ transplants patients (Blaheta *et al.*, 2000).

CLINICAL ASPECTS OF MPA PK/PD DATA

Three large, double-blinded, randomized trials have shown that the addition of MMF to another immunosuppressants results in a significant reduction in the rate of biopsy-proven acute rejection during the first 6 months after renal transplantation. Although MMF was initially used in renal transplant recipients in 1~3 g daily dose without routine trough level monitoring, the size of the reduction in incidence and severity of acute rejection episodes for patients with 2 or 3 g MMF was similar; however, the 3 g dose was less tolerated and dose-related reduction in the incidence of rejection was noted (Sollinger, 1995; Tricontinental trial, 1996; Halloran *et al.*, 1997; Sollinger *et al.*, 1992). Therefore, the current daily-recommended dose is 2 g (Lipsky, 1996).

In spite of the various clinical circumstances represented in three multicenter trials, a consistent picture emerges (Table I). Some pharmacokinetic-pharmacodynamic relationships of MMF in the organ-transplant patients have already been reported (Sollinger, 1995; Kobashigawa *et al.*, 1998; Shaw *et al.*, 2001; Hale *et al.*, 1998; Nicholls, 1998). In the kidney transplant recipients given MMF at a fixed dose (2 g/day), as a part of a triple regimen including cyclosporine A (CsA) and steroid, pharmacokinetic parameters are highly variable in adult and pediatric patients and it also showed some association between MPA-$AUC_{0\text{-}12hrs}$ or MPA trough level and graft function and the percentage of free MPA level but not the total MPA trough level negatively correlated with erythrocyte as well as leukocyte count (Weber *et al.*, 1998). Therefore, a fixed dose regimen of MMF might no longer be the best approach for the management of transplants patients (Cattaneo *et al.*, 2001).

The MMF dose as well as MPA trough levels correlated with the hemoglobin level (van Besouw *et al.*, 1999). The MPA-AUC has been shown to be the best predictor of MMF suppressive effects on rejection in renal transplantation and a sigmoid relationship between MPA-AUC and the probability of rejection has been shown (Hale *et al.*, 1998; Nicholls, 1998). On the basis of both safety and efficacy, a dose of 1 g of MMF twice a

Table I. Biopsy Proven Rejection (BPR) Rates and Treatment Failure Rates (TFR) in the Three Primary Controlled Double-Blind Trials of MMF Renal Transplantation

	Placebo	AZT	MMF 2 g	MMF 3 g
US trial (Sollinger, 1995)		N=166	N=167	N=166
BPR		38.0%	19.8%	17.5%
TFR		47.6%	31.1%	34.8%
Tricontinental trial (1996)		N=166	N=173	N=164
BPR		35.3%	19.7%	17.5%
TFR		50.0%	38.2%	34.8%
European trial (1995)	N=166		N=165	N=160
BPR	46.4%		17.0%	13.8%
TFR	56.0%		30.3%	38.8%

#AZT, azathioprine; MMF, mycophenolate mofetil

daily represents the recommended starting dose and dose adjustment for optimization of efficacy should take account of the increase in MPA-AUC that occurs with time following renal transplantation. There was no overall improved efficacy over 2 g dose daily and the tolerability in patients receiving 2 g dose daily is better than 3 g dose daily (Fulton and Markham, 1996). Although increased doses are clearly associated with greater likelihood of common adverse events, plasma MPA levels appear to be a relatively poor indicator of its adverse events (Nicholls, 1998).

The MPA-C_{max} in patients with hemodialysis (HD) or peritoneal dialysis (PD) was lower than those with normal renal function, but mean MPA-AUC in dialysis patients are similar to expected values for subjects with normal renal function (MacPhee *et al.*, 2000). The mean MPA-C_{max} was about 11.5 mg/L for PD patients and 16.0 mg/L for HD patients, in close agreement with a value of 16.1 mg/L found for HD patients, with clear evidence of accumulation in a single patient studied on a maintenance dose (MacPhee *et al.*, 2000; Johnson *et al.*, 1998). It has also been noted in renal transplant recipients with variable renal function that MPAG-AUC and MPA-C_{max} increased, but there is no significant difference in MPA-AUC and T_{max} as GFR decreased (Johnson *et al.*, 1998). The MPAG-AUC following a single dose was approximately five times higher in patients with dialysis than normal renal function (MacPhee *et al.*, 2000; Johnson *et al.*, 1998; Bullingham *et al.*, 1996). The associations between MPA-AUC and the risk for acute rejection were first noted in a retrospective PK analysis and in cohort study about adult renal transplant patients (Nicholls, 1998; Takahashi *et al.*, 1995).

In clinical trial of the safety and efficacy of oral MMF for the prevention of acute rejection after renal transplantation, MPA-AUC and trough level are significantly related to the its efficacy like incidence of biopsy-proven rejection, whereas MMF dose is significantly related to tolerability and adverse events (van Gelder *et al.*, 1999). A significant correlation has been reported between trough level and MPA-AUC within 3 weeks after renal transplantation (Wollenberg *et al.*, 1998; Pirsch and Sollinger, 1996) and between MPA-AUC and the probability of rejection (Sollinger *et al.*, 1992; Takahashi *et al.*, 1995; Shaw and Nowak, 1995). Although in a small group of patients, MPA trough levels were significantly related to the occurrence of side effects (Smak Gregoor *et al.*, 1998). Even though the MPA-AUC and trough levels can help predict the risk of rejection following solid-organ transplantation, MPA-AUC is a more powerful univariate predictor of rejection because of the greater inter-patient and the intra-patient variability of trough levels (DeNofrio *et al.*, 2000; Shaw *et al.*, 2001)(Table II).

The MPA-AUC may be range more than 10-folds in patients who receive oral 2 g daily of MMF. The reasons of the inter-individual variability in MPA-AUC values may be inter-individual variability of MPA-PK (Bullingham *et al.*, 1998), drug-drug interactions (Bullingham *et al.*, 1996; Zucker *et al.*, 1997 & 1999; Smak Gregoor *et al.*, 1999), perturbations of MPA-AUC values in various disease states (Weber *et al.*, 1998; Kaplan *et al.*, 1998 & 1999) and MPA free fraction (Shaw *et al.*, 2001). Therefore, because of the extent of patient variability in MPA rejection profile, routine MPA monitoring would favor to balance effective immunosuppression against opportunistic infections after organ transplantation. These MPA-PK studies support an association between lower MPA levels and increased allograft rejection and it may provide important information to help the clinician master the balance between excessive or inadequate immunosuppression in the individual

Table II. Intraindividual % CV for MPA predose level and MPA-AUC (van Gelder *et al.*, 1999; Shaw *et al.*, 2000; Weber *et al.*, 1999)

N	Predose level (mg/L)		AUC(mg-hr/L)		Comments
	Mean % CV	% CV range	Mean % CV	%CV range	
20	44	19-84	30	13-45	Renal transplant patients with stable renal function in first 3 months post-surgery
13	55	30-118	47	20-80	Renal transplant patients with impaired early renal function in first 3 months post-surgery period
19	43	25-77			Renal transplant patients receiving maintenance therapy
15	43	30-61			Renal transplant patients during first post-surgery month
36	62	28-116			Liver transplant patients during first post-surgery month
150		40-60		17-40	Renal transplant patients during the first 6 months following transplant surgery
17	Median C_{min}=1.8	Range (%) =22-198	Median AUC =64.3	Range (%) = 33-133	Renal transplant patients with stable renal function during the first 6 months
7	4- to 18-fold range of predose trough levels during the first 50 post-transplant days for liver transplant recipients				

transplant patients (DeNofrio *et al.*, 2000). The fact that increasing dose-interval MPA-AUC lowers the risk for acute rejection is based on retrospective reviews of clinical studies in kidney and heart transplant patients and on the multicenter prospective trial in which the enrolled kidney transplant patients had their MMF doses adjusted to achieve and maintain one of three MPA-AUC values for 6-months period (Kobashigawa *et al.*, 1998; Hale *et al.*, 1998; DeNofrio *et al.*, 2000; Shaw *et al.*, 2000; Oellerich *et al.*, 2000).

An analysis of MPA-PK data developed in a multicenter study of pediatric renal transplant patients concluded that a target range of 30 to 60 μg-hr/ml provides for significant reduction in acute rejection and would assure avoidance of chronic exposure to high MPA concentrations. Therefore, the MPA-AUC is predictive of the likelihood of allograft rejection after renal transplantation in patients receiving MMF (Hale *et al.*, 1998; Oellerich *et al.*, 2000). As well as there are a significant correlation between MPA trough level and MPA-AUC in renal transplant recipients (Wollenberg *et al.*, 1998; Pirsch and Sollinger, 1996) and between MPA-AUC and the probability of rejection (Sollinger *et al.*, 1992; Takahashi *et al.*, 1995; Wollenberg *et al.*, 1998; Pirsch and Sollinger, 1996; Shaw *et al.*, 1995; Vanrenterghem, 1997) the probability of rejection decreased as the natural log of MPA-AUC increased (Shaw *et al.*, 1995). In the correlation between acute rejection rate and MPA-AUC, fitted to a logistic curve with MPA-AUC plotted on a logarithmic scale, a good fitting is obtained, such that, as MPA-AUC increases, the probability of the occurrence of acute rejection decreases (Bullingham *et al.*, 1996). In addition to standard CsA monitoring, the 4-points sampling strategy to predict MPA-AUC in the first week posttransplant period, which may facilitate optimization of MMF at a time when patients are most vulnerable to acute rejection (Pillans *et al.*, 2001).

There were no significant differences in the incidence of rejection episodes, number of rejection episodes, GFR at 6 and 12 months, and allograft or patient survival between pediatric patients receiving MMF versus AZT (Benfield *et al.*, 1999). This result is in contrast to that reported in adults receiving renal allografts in the treatment with MMF (Sollinger HW, 1995; Tricontinental trial, 1996; European trial. 1995). In pediatric renal recipients who treated with concomitant with CsA and steroids, both low MPA-AUC as well as low predose MPA levels is significantly associated with the risk of acute rejection (Bullingham *et al.*, 1998; Weber *et al.*, 1999; Armstrong *et al.*, 2001) and provides that MMF shows highly variable inter-individual and intra-individual PK and PK/PD relationships

Table III. Acute rejection rate outcomes for the MMF multicenter randomized concentration-controlled trial in renal transplant patients (Vanrenterghem, 1997)

Group	Target AUC	Rejection rate (%) *
Low	22.1	25.5
Intermediate	40.9	8.5
High	64.2	5.8

* Incidence of biopsy-proven rejection

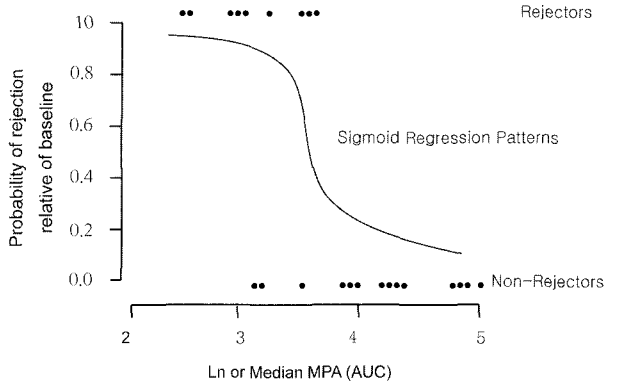

Fig. 1. Schematic relationship diagram between efficacy and plasma MPA-AUC in renal transplant patients (Hale *et al.*, 1998; Bullingham *et al.*, 1996)

between both MPA-AUC as well as MPA trough levels (Hale *et al.*, 1998; Weber *et al.*, 1998 & 1999; Oellerich *et al.*, 2000) and risk of acute rejection have been reported in the renal transplant recipients(Hale *et al.*, 1998; Bullingham *et al.*, 1996; Vanrenterghem, 1997)(Table III; Fig. 1)

Although a full 12-hrs MPA-AUC provides the most accurate assessment of risk for acute rejection compared to other strategies such as predose or abbreviated sampling estimations of exposure, it is impractical to physician and inconvenient to patient. Therefore, abbreviated sampling strategies for estimation of the full 12-hrs MPA-AUC and the use of single timed sample such as the predose trough or one sample at a specific time following a dose of MMF are need to further investigate in ongoing studies (Shaw *et al.*, 2001). Serial daily determinations of predose MPA levels were used for individualization of MMF doses in heart transplant patients receiving concomitant tacrolimus (Meiser *et al.*, 1999). Although a single predose MPA levels provides a less precise estimation of the risk for acute rejection, serial daily predose estimations will provide a reasonable alternative to the full 12-hrs MPA-AUC. In Asian renal transplant recipients, there is no significant difference in trough level, C_{max}, and T_{max} of MPA among the three different periods such as 1 week, 1 month, and 3 month. However, MPA-AUC

increased significantly with time and mean percentage increases were 22.9% at 1 month and 62.4% at 3 month compared to 1 week (Yeung *et al.*, 2000).

Even if there is a role for drug monitoring of MMF for considerable individual variations in PK parameters of MPA, measuring full MPA-$AUC_{0\text{-}12hrs}$ are laborious, time consuming and needs multiple bloods sampling from patients and are costly. To overcome these problems, limited sampling strategies (LSS) have been proposed for CsA in which two or three points are determined (Keown *et al.*, 1996). Therefore, several studies about LSS for estimating the full MPA-$AUC_{0\text{-}12hrs}$ in various organs transplanted patients at several periods after transplantation (Table IV). The abbreviated AUC by three-time points strategy such as 0;1;2 hrs or 0;0.5;

2 hrs measurement of MPA level has been shown to have good correlation to actual full MPA-$AUC_{0\text{-}12\ hrs}$ in patients with stable renal function (Yeung *et al.*, 2001; Pawinski *et al.*, 2002).

An intravenous formulation of MMF for patients unable to tolerate oral medications has been recently introduced. MMF is also rapidly hydrolyzed within a few minutes to MPA after intravenous administration (Lee *et al.*, 1990). In PK studies after MMF dose to normal volunteers and to renal and hepatic transplants recipients in the immediate postoperative period, MPA-C_{max} was higher after intravenous than oral dosing. The MPA-$AUC_{0\text{-}24hrs}$ after intravenous dosing was significantly higher than oral administration, but the total AUC was statistically equivalent and T_{max} was similar (Bullingham *et al.*, 1998 & 1996). The intravenous MMF 1 g twice a day should provide efficacy at least equivalent to oral MMF regimen without increased toxicity, and intravenous dosing provides an acceptable alternative regimen in those patients unable to take oral medication in the immediate transplant period (Pescovitz *et al.*, 2000).

Several human cardiac allograft clinical trials with MMF as an immunosuppressant have been promising (DeNofrio *et al.*, 2000; Taylor *et al.*, 1994; Kirklin *et al.*, 1994; Ensley *et al.*, 1993). In a randomized, prospective clinical trial about heart transplanted recipients (Kobashigawa *et al.*, 1998), MMF has shown equivalency in efficacy for preventing rejection, but, it does appear to be superior based one and three years survival results when compared to AZT. Several recent studies have demonstrated that blood MPA levels may be predictive of the likelihood of allograft rejection after renal and heart transplantation (DeNofrio *et al.*, 2000; Meiser *et al.*, 1999; Shaw *et al.*, 1999; Hale *et al.*, 1998). A relationship exists between MPA trough levels and allograft rejection in patients receiving a tacrolimus-based immunosuppressive regimen and therapeutic drug monitoring using MPA trough levels appears to be associated with suppression of acute myocardial rejection in heart transplanted recipients (Meiser *et al.*, 1999). In the study about efficacy of MMF in preventing the recurrence of acute rejection following an initial rejection episode in renal transplant patients who characterized histological mild or moderate rejection in the first year after transplantation, the addition of MMF to maintenance therapy may prevent recurrent rejection episodes in the subsequent follow-up year (Vasquez *et al.*, 2001).

One of the most controversial issues about immunosuppression in the organ transplantation is whether the increased risk of acute rejection that may be associated with calcineurin inhibitor-sparing regimens is outweighed by the metabolic benefits gained (Land *et al.*, 2001). Replacement of CsA with AZT can lead to an increase in the rate of acute rejection without a negative impact on long-term outcome (MacPhee *et al.*, 1998; Hollander *et al.*, 1995). Because MMF has a more immuno-

Table IV. Multiple regression analysis to correlate abbreviated MPA-AUC values with actual full MPA-$AUC_{0\text{-}12hr}$

N	Sampling time(hr)	Model equation for abbreviated AUC	R^2	Comments
21	0;0.5;1	$10.88+8.18C_{0hr}+0.66C_{0.5hr}+0.76C_{1hr}$	0.810	Adult renal transplant patients treated with tacrolimus at various period after transplantation (Pawinski *et al.*, 2002)
	0;1;2	$10.32+6.72C_{0hr}+0.81C_{1hr}+1.87C_{2hr}$	0.772	
	0;0.5;2	$7.75+6.49C_{0hr}+0.75C_{0.5hr}+2.43C_{2hr}$	0.862	
10	1.25;2;10	$15.93+0.73C_{1.25hr}+0.8C_{2hr}+7.32C_{10hr}$	0.861	Adult renal transplant patients treated with CsA and prednisolone at various period after transplantation (Yeung *et al.*, 2001)
	0;1;2	$15.19+6.92C_{0hr}+1.08C_{1hr}+0.72C_{2hr}$	0.756	
	1.25;2;4;10	$10.72+0.94C_{1.25hr}+0.84C_{2hr}+1.46C_{4hr}+6.5C_{10hr}$	0.901	
	0;1;2;4	$6.02+5.61C_{0hr}+1.28C_{1hr}+0.9C_{2hr}+2.54C_{4hr}$	0.890	
10	0;1;2	$25.55+5.16C_{0hr}+0.76C_{1hr}+0.46C_{2hr}$	0.966	Adult renal transplant patients treated with CsA and prednisolone at 3rd month period after transplantation (Yeung *et al.*, 2000)
10	0;1;3;6	$9.02+3.77C_{0hr}+1.33C_{1hr}+1.68C_{3hr}+2.96C_{6hr}$	0.836	Adult renal transplant patients within the first month after transplantation (Johnson *et al.*, 1999)

suppressive effect than AZT (Sollinger HW, 1995; Tricontinental trial, 1996; Mathew, 1998; US trial, 1999), many centers are now attempting calcineurin inhibitor sparing with MMF. Numerous studies in which CsA was completely or partially replaced by MMF have reported that the metabolic benefits of increased renal function and decreased hypertension are shown without increased rates of acute rejection (Ducloux *et al.*, 1998; Houde *et al.*, 2000).

The long-term MMF monotherapy has been reported as an optimal, safe, and effective immunosuppressive maintenance regimen in cadaveric kidney transplantation (Land *et al.*, 2001). MMF has been used also in antoimmune diseases, including rheumatoid arthritis, systemic vasculitis and autoimmune hematolytic anemia (Goldblum, 1993; Nowack *et al.*, 1999; Zimmer-Molsberger *et al.*, 1997). In the NZB/W mouse, which develops an immune complex disease with nephritis that is similar to human systemic lupus erythematosus (SLE) and patient with human SLE refractory to other immunosuppressive agents, MMF appears to be a safe and effective alternative immunosuppressant for both renal and non-renal disease in human SLE (Corna *et al.*, 1997; Karim *et al.*, 2002).

CLINICAL TOXICITY OF MPA

MPA can cause toxicity, mainly of hematological and/or gastrointestinal nature. The risk for developing side effects related to MPA concentration has not been clear to establish. The occurrence of gastrointestinal toxicity is not related to the plasma MPA or MPAG level (Shaw *et al.*, 1998; Bullingham *et al.*, 1998; Oellerich *et al.*, 2000). The highest frequency of side effects in the multicenter concentration-controlled study occurred in the highest target MPA-AUC group (Hale *et al.*, 1998) and there is a significant association between free MPA-AUC and the risk for severe side effects (van Gelder *et al.*, 1999; Oellerich *et al.*, 2000). Patients with MPA-AUC appear to be at high risk for graft rejection, whereas high-target MPA-AUC and concentrations can increase toxicity (Table V).

MMF efficacy, as part of maintenance immunosuppressive therapy in the prevention of acute renal graft rejection, has already been demonstrated in several studies that have shown the superiority of daily dose of 2 g MMF compared with AZT or placebo regarding positive, biopsy-confirmed acute rejection and safety when combined with CsA(Sollinger, 1995; Tricontinental trial, 1996; European trial. 1995; Meier-Kriesche *et al.*, 2000).

Even with an optimal dose of 2 g per day, side effects such as hematological disorder have been observed, leading to dose reduction or temporary drug withdrawal in some cases. In the study designed on a CsA based immunosuppressive regimen, it clearly demonstrates a PK-PD relationship between MPA level and side effects or acute rejection at a fixed dose of 2 g/day. A low MPA-$AUC_{0\text{-}12hrs}$ were obtained after a reduction in oral dose in patients who had experienced MMF-related side effects was associated with a high risk of rejection. In patients with low MPA-AUC, acute rejection was more frequent than in those with high MPA-AUC and meant the relatively poorer predictive value of the MPA-C_{min} compared to MPA-AUC (Kobashigawa *et al.*, 1998). On the other hand, some study clearly demonstrated that both the MPA-C_{min} and MPA-AUC are associated with the incidence of acute rejection but not with side effects (Hale *et al.*, 1998; van Gelder *et al.*, 1999).

In other study, both the MPA-C_{min} and MPA-AUC were higher in patients who presented with side effects than those with uneventful outcomes; however, only MPA-AUC shown statistical significance. Moreover, the MPA-C_{30min} was significantly associated with an increased risk of side effects at 2 g dose daily than MPA-$AUC_{0\text{-}12hrs}$ and high MPA-C_{30min} values would explain the occurrence of side effects in patients with MPA-AUC within nontoxic limits (Mourad *et al.*, 2001). In contrast, MMF safety is related more to the oral dose than to the MPA-PK parameters such as C_{min}, C_{max}, and AUC. However, it should be mentioned that there was a distinct preponderance of gastrointestinal side effects compared with other MMF-related adverse effects such as hematological disorders (van Gelder *et al.*, 1999; Hale *et al.*, 1998). The high incidence of hair loss is remarkable a side effect (Smak Gregoor *et al.*, 1998), which was not found in the three large randomized trials (Tricontinental trial, 1996; Sollinger, 1995; European trial,

Table V. Incidence of adverse events (AE) leading to withdrawal in the three target MPA-AUC groups (van Gelder *et al.*, 1999)

	Target MPA-AUC Group			
	Low	Intermediate	High	Total
Number of patients	51	47	52	150
Withdrawal due to AE[a]	4(4.7%)	11(23.4%)	23(44.2%)	38(25.3%)
Diarrhea	0	2(4.3%)	5(9.6%)	7(4.7%)
Vomiting	0	1(2.1%)	3(5.8%)	4(2.7%)
Abdominal Pain	0	0	4(7.7%)	4(2.7%)
Leukopenia	1(2.0%)	1(2.1%)	9(17.3%)	11(7.3%)
Pneumonia	0	3(6.4%)	2(3.8%)	5(3.3%)

[a]Data concern the number of patients with one or more adverse events leading to dicontinuation of the study medication. Only the most frequent adverse events are shown.

1995). In these trials, MMF was only given in combination with CsA and prednisone and there are clear differences in plasma MPA levels between MMF-treated patients with or without CsA. The MPA concentrations were significantly high in non-CsA treated patients, possibly due to increased glucuronidation as a result of CsA-induction of the CYP450 complex (van Gelder *et al.*, 1999; Smak Gregoor *et al.*, 1998). There is limited experience-using MMF in human liver transplant recipients or in combination with tacrolimus and MMF may be useful in controlling rejection and drug toxicities in liver transplanted patients under tacrolimus and steroids. However, it should be used with caution in patients with HCV because it may be contributory to HCV recurrence (Zucker *et al.*, 1997; Gavlik *et al.*, 1997; Klintmalm *et al.*, 1993). The use of MMF at 3 years was associated with a slightly higher incidence of gastrointestinal and hematologic adverse events and a slightly higher infection and malignancy in cadaveric renal transplantation (Table VI).

THERAPEUTIC DRUG MONITORING STRATEGIES OF MPA

MMF, a prodrug of MPA, was initially described as having a moderate inter-individual PK variability (Bullingham *et al.*, 1996), leading to predictable plasma levels after a given dose. However, numerous sources of variability have been existed and providing the basis for routine therapeutic monitoring (Shaw *et al.*, 2001 & 2000; Weber *et al.*, 1999; Johnson *et al.*, 1999). Therapeutic MMF monitoring should take into account factors that may affect absorption, metabolism, and MPA free-fraction rate. The PK-PD relationships between MPA-AUC as well as predose MPA levels and the risk of acute rejections have been established in pediatric and adult renal transplant recipients (Oellerich *et al.*, 2000; Weber *et al.*, 1999; van Gelder *et al.*, 1999; Hale *et al.*, 1998). Both low MPA-$AUC_{0\text{-}12\ hrs}$ as well as low predose levels is significantly associated with the risk of acute rejection in pediatric renal transplant recipients (Weber *et al.*, 1999). These results imply that MPA monitoring in clinical practice can further minimize the risk of acute rejection during early posttransplantion period (Armstrong *et al.*, 2001).

Monitoring of plasma MPA after kidney transplantation in patients receiving an immunosuppressive regimen combining tacrolimus and MMF demonstrates the relationship between plasma MPA levels and toxicity. The pharmacokinetic strategy would optimize MMF therapy in kidney transplant patients and also support the need for MPA therapeutic monitoring (Mourad *et al.*, 2001). In routine clinical practice, the MPA-C_{min} and

Table VI. Principal Adverse Events (Mathew, 1998)

		AZAT (n=162)	MMF 2 g (n=171)	MMF 3 g (n=164)
		19.8%	35.1%	38.4%
Gastrointestinal	Diarrhea			
	Abdominal Pain	25.9%	29.8%	34.1%
	Nausea	21.6%	19.3%	24.4%
	Vomiting	7.4%	16.4%	19.5%
Hematological	Leukopenia	30.9%	19.9%	37.8%
	Thrombocytopenia	12.3%	8.8%	6.1%
	Anemia	9.3%	15.8%	11.6%
Malignancies	Lymphoma/LPD	0.6%	1.2%	1.8%
	Nonmelanoma skin carcinoma	13.6%	11.1%	4.3%
	Other	3.7%	2.3%	5.5%
Infections	CMV viremia/syndrome	12.3%	12.9%	13.4%
	CMV tissue invasive	6.8%	7.0%	11.0%
	Herpes simplex	24.1%	22.2%	26.8%
	Cutaneous Herpes zoster	9.3%	8.2%	11.6%
	Disseminated/visceral Herpes zoster	<1.0%	0	1.2%
	Peumocystis	1.9%	<1.0%	0
	Aspergillus/Mucor Tissue-invasive	<1.0%	<1.0%	<1.0%
	Disseminated *Candida* Fungemia	0	<1.0%	0
	Tissue-invasive *Candida* Fungemia	<1.0%	0	0

AZT, azathioprine; MMF, mycophenolate mofetil; LPD, lymphoproliferative disorders; CMV, cytomegalovirus

Table VII. Prospective PK and TDM plan of MPA in renal transplant patients (Shaw *et al.*, 1998)

1. Each patient is enrolled for a 90-day period.
2. MMF dosage strategies to achieve target MPA AUC or predose concentration values were developed based on earlier PK-PD studies.
3. Five 2-hour (1 predose and 4 postdose timed plasma samples) abbreviated MPA AUC assessments are made in the morning after an overnight fast at 4, 7, 11-14, 28, and 90 days after renal transplant surgery in all study patients.
4. Validated HPLC methods are used for MPA and MPAG measurement: free fraction of MPA is measured at each scheduled study day with a validated method for measuring free MPA concentration.
5. Dosing decisions are made on the afternoon of the same day that the timed samples are obtained and analyzed.
6. Half of the study patients are randomized to have dosing decisions made on the basis of the abbreviated MPA-AUC; dosing decisions for the other half are based on predose MPA concentration.

At the conclusion of this study the interpatient variances (%CV) of MPA-AUC will be determined for the AUC-based and predose concentration subgroups to determine which approach gave the lowest within-patient MPA variance. The incidence of acute rejection and side effects will be determined.

C_{max} during the first hour after oral dose ($C_{30\ min}$, C_{60min}) show a significant correlation with the occurrence of adverse effects as indicated by the $AUC_{0\text{-}12hrs}$ in the prospective study to assess the PK-PD relationship for MPA in kidney transplant patients receiving low-dose MMF about 500 mg twice a day in combination with tacrolimus.

Treatment with MMF may reduce the incidence and severity of adverse effects in pediatric renal transplant recipients, but its use is currently laden with a high incidence of adverse effects. Because there exists a significant individual variability in MPA-AUC in organ transplant patients, MPA-PK should be evaluated in all patients, particularly in those with impaired renal function and dose adjustments may be necessary (Butani *et al.*, 1999). Because of great variations in MPA-AUC in the early posttransplant period when the risk for acute rejection is greatest and shows good correlation between MPA-AUC and risk for acute rejection, measurement of MPA-AUC can be valuable in order to establish the adequacy of MPA levels to avoid risk for acute rejection and to appropriate dose of MMF to avoid side effects in this period and to establish a baseline for dose reduction of other concomitant immunosuppressive drugs for maintenance immunosuppression (Shaw *et al.*, 1998). Therefore, therapeutic MPA-PK monitoring might contribute to decrease the risk of MMF-related toxicity (Cattaneo *et al.*, 2001). In study about MMF monitoring in kidney-transplanted patients, some serious toxic effects were observed at C_{min} above 4 μg/ml, whereas there were some cases of interstitial rejection at C_{min} below 2 μg/ml. There was also a negative correlation between dosage and body weight, suggesting dosages related to body weight might be better than fixed ones (Brusa *et al.*, 2000). Routine therapeutic monitoring of MMF may assist in the management of cardiac transplant recipients during the first year post transplant when the highest incidence of rejection occurs (Yamani *et al.*, 2000). A fixed dose regimen of MMF might no longer be the best approach for the management of organ transplant patients. Whether optimizing MMF dose by monitoring MPA levels may help to maintain graft function in the long term is an intriguing possibility arising from present findings such as an association between MPA-AUC or MPA trough level and graft function (Cattaneo *et al.*, 2001).

DRUG-DRUG INTERACTIONS OF MPA

The effect of concomitant medications on the PK of MPA have been revealed that the primary sites of interactions and effects are decreased absorption in the gastrointestinal tract, inhibition of enterohepatic cycling and inhibition of transport of glucuronide (Table VIII). The use of antacids decrease both the MPA-AUC and MPA-C_{max} by decreasing MMF absorption and intake of food also influences the PK of MMF, decreasing the MPA-C_{max} by -25%, without affecting MPA-AUC (Bullingham *et al.*, 1996). An increase in MPA-C_{min} and MPA-AUC when tacrolimus is used in conjunction with MMF has been reported (Zucker *et al.*, 1997). The MPAG-AUC is lower in patients receiving tacrolimus than CsA in combination with MMF at the same dose of 2 g/day. Recently, it has been demonstrated that CsA affects the MPA-C_{min}, which is lower in patients receiving CsA than in patients not receiving CsA at the same MMF dose, showing less inter-individual variations (Smak Gregoor *et al.*, 1999). Metabolic interactions among CsA, 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 esterase, but could be related to a possible inhibitory effect of tacrolimus or stimulatory effect of CsA on MPA metabolism.

Human serum albumin, high concentrations of MPAG, and high doses of sodium salicylate significantly affect albumin MPA binding (Nowak and Shaw, 1995). Because early acute renal dysfunction after transplantation such as acute tubular

Table VIII. Mycophenolate Drug-Drug Interactions (Facts and Comparisons® St. Louis, 2003)

Drugs	Interactions and comments
Acyclovir, Ganciclovir(Neyts *et al.*,1998)	MPAG and acyclovir plasma AUCs were increased 10.6% and 21.9%, respectively and the potential for 2 drugs to compete for tubular secretion.
Antacids (Bullingham *et al.*, 1996)	Absorption of a single MPA dose was decreased. The C_{max} and AUC for MPA were 33% and 17% lower, respectively.
Azathioprine	It is recommended to avoid concomitant use because of a lack of clinical studies.
Cholestyramine (van Gelder *et al.*, 2001)	Decreased absorption of MPA in the gastrointestinal tract and MPA AUC decreased about 40%.
CsA(van Gelder *et al.*, 1999)	Suppression of export of MPAG into bile and enterohepatic cycling of MPA; Decreased MPA - AUC, increased MPAG- AUC.
Iron	MPA absorption and MPA- AUC were significantly decreased.
Metronidazole	Suppression of enterohepatic cycling of MPA by inhibition of anaerobic bacteria and glucuronidase
Probenecid	In animals, 3-folds increase in plasma MPAG
Salicylate	Increased the free fraction of MPA

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, leading to an increase in MPA free fraction, presumably by a competitive displacement mechanism (Shaw *et al.*, 1998).

Because of higher glucuronidation of MPA in male than female recipients of a cadaveric renal allograft and under immunosuppressive therapy with MMF, gender differences and cotreatment with tacrolimus must be taken into consideration (Morissette *et al.*, 2001). It shows clearly that the CsA-AUC achieved by concomitant CsA is determining factor for the MPA-AUC because of CsA-induced inhibition of MPA glucuronidation (Filler *et al.*, 2001) and suggest that MPA dose must be reduced to maintain MPA-AUC when lowering the CsA-AUC. The MPA-$AUC_{0\text{-}12hrs}$ and trough levels display a progressive significant increase during the first 3 months after transplantation, suggesting a change in the MPA-PK during this period. These changes have been described previously in adult and pediatric renal transplant populations (van Gelder *et al.*, 1999; Weber *et al.*, 1999). 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 (Weber *et al.*, 1999). Factors influencing MPA metabolism may include protein binding (Vanrenterghem, 1997), changes in enterohepatic recirculation, and induction of hepatic glucuronyl-transferase activity by glucocorticoids (Zhu *et al.*, 1996).

SUMMARY

Even if PK of MPA has been showed extensive inter-individual variability, there is a significant relationship between the full 12-hrs MPA dose interval AUC and the risks for acute rejections and hematological side effects based on retrospective investigations in renal and heart transplants patients and on prospective studies in renal transplants patients.

Recent clinical investigations suggested that improved effectiveness and tolerability will results from the incorporation of MPA therapeutic drug monitoring into routine clinical practice, providing effective MMF dose individualization in renal and heart transplant patients. However, routine measurement of the full 12-hrs interval MPA-AUC is very inconvenient to patient and very impractical and would be cost-prohibitive. To overcome these problems, limited sampling strategies have been proposed for MPA-AUC determination in which three or four times are determined. The limited sampling strategy was first introduced in 1987 to minimize the number of plasma samples necessary to estimate AUC values of various drugs. This approach has proven to be effective, validated tool in monitoring efficacy and toxicity of drugs while minimizing the number of blood samples. Among these strategies, a reliable model based on three samples obtained within the first 2 hrs after dose of MMF is convenient to both inpatient and outpatient and is cost effective and applicable to most clinical settings.

Although it may be have a practical role in therapeutic drug monitoring of MPA in organ transplant patients taking MMF as part of the immunosuppressive drugs, caution is performed when using limited sampling strategies for the predictions of MPA-AUC in transplants patient. Also, there is a need for carefully designed prospective investigations of therapeutic drug monitoring protocols or guidelines in order to determine the best sampling times for organ transplanted populations in various clinical settings.

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