Individualisation of mycophenolate mofetil therapy

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INDIVIDUALISATION OF MYCOPHENOLATE MOFETIL THERAPY

Explaining variability in mycophenolic acid pharmacokinetics and introducing therapeutic drug monitoring

Individualisation of Mycophenolate Mofetil Therapy

Explaining variability in mycophenolic acid pharmacokinetics and introducing therapeutic drug monitoring

Individualisering van de mycophenolate mofetil therapie

Verklaring van variabiliteit in de farmacokinetiek van mycofenolzuur
en introductie van therapeutic drug monitoring

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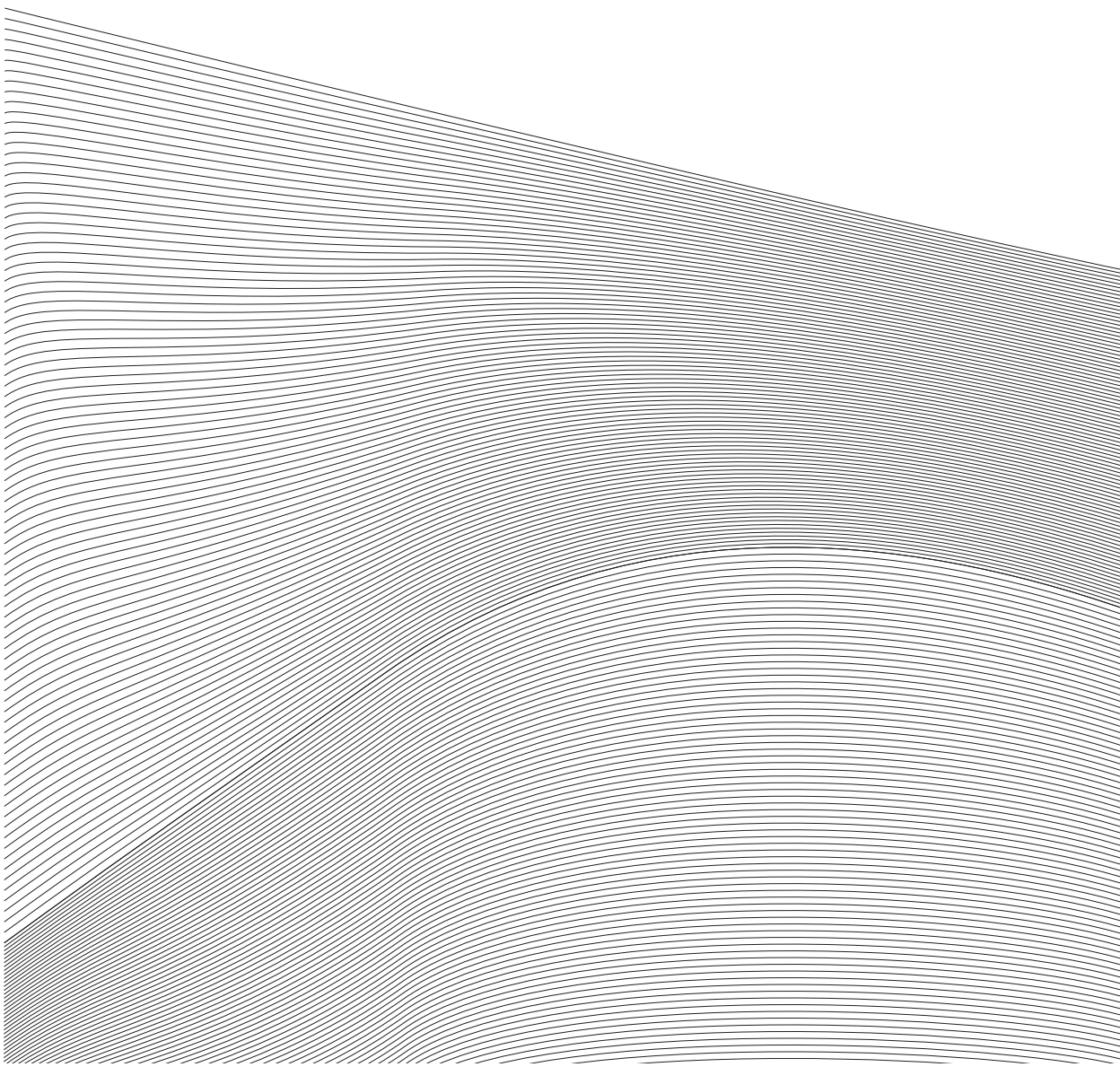
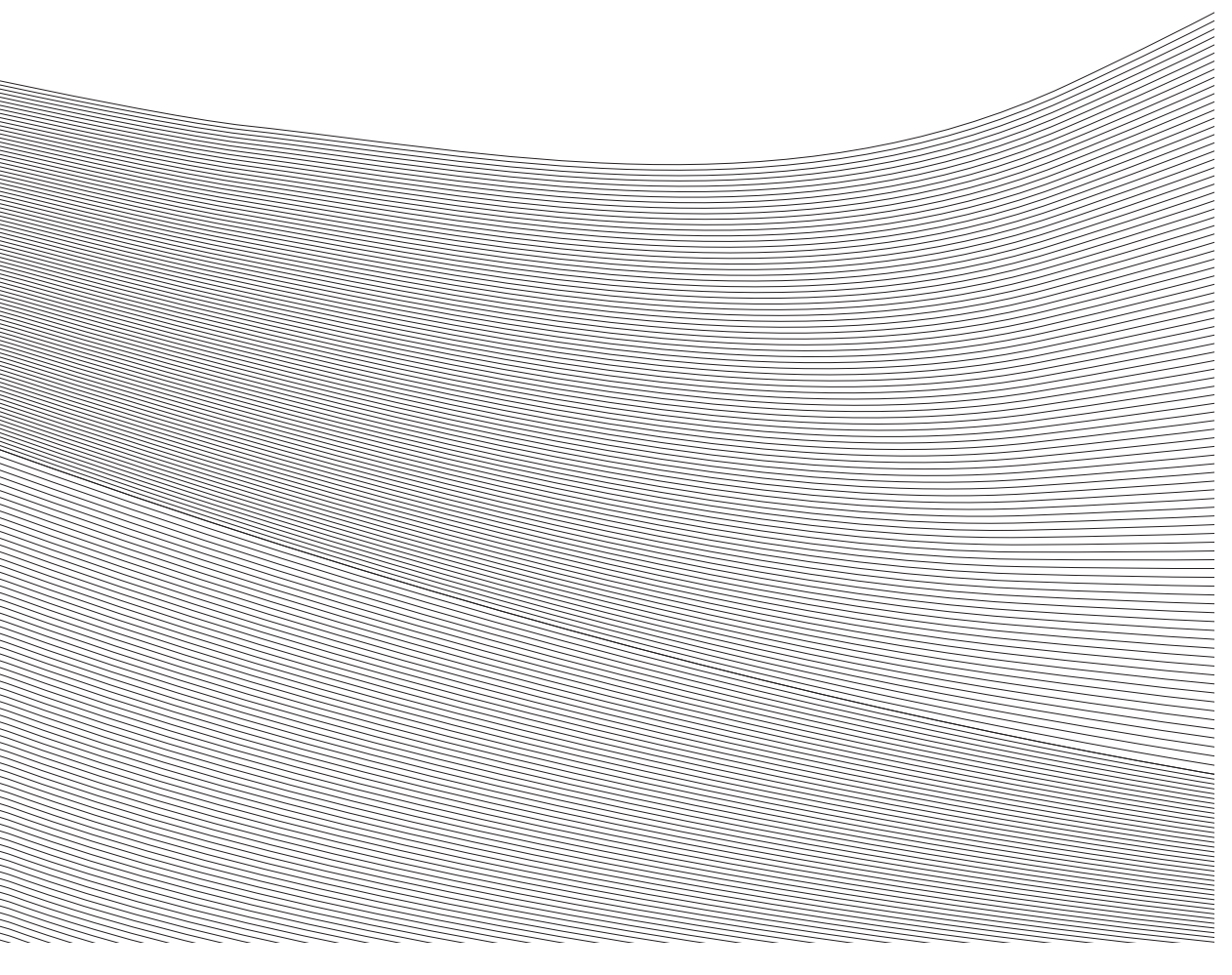
INDEX



Chapter 1

INTRODUCTION



Chapter 1.1

Why Individualise Mycophenolate Mofetil Dose in Renal Transplant Recipients, and How?

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1 ABSTRACT

The immunosuppressive agent mycophenolate mofetil has been succesfully used over the past 10 years to prevent acute allograft rejection after renal transplantation. It has mainly been administered with a fixed dose of mycophenolate mofetil 1000 mg twice daily. The pharmacokinetics of mycophenolic acid, the active moiety of the prodrug mycophenolate mofetil, show large between-patient variability, and exposure to mycophenolic acid correlates with the risk for acute rejection. This suggests that the already excellent clinical results can be further improved by mycophenolate mofetil dose individualisation. This review discusses different arguments in favour of individualisation of mycophenolate mofetil dose, as well as strategies for managing mycophenolate mofetil therapy individualisation, including pharmacokinetic and pharmacodynamic monitoring and dose individualisation based on pharmacogenetic information. It is expected that pharmacokinetic monitoring of mycophenolic acid will offer the most effective and feasible tool for mycophenolate mofetil dose individualisation.

2 INTRODUCTION

Mycophenolate mofetil (MMF) is a prodrug of mycophenolic acid (MPA). The recently introduced enteric-coated mycophenolate sodium (EC-MPS) contains MPA as the active compound. MPA has immunosuppressive properties through the selective, reversible and non-competitive inhibition of inosine monophosphate dehydrogenase (IMPDH). Inhibition of this enzyme, which consists of two isoforms (type I and type II), leads to an arrest of synthesis of guanosine monophosphate, which is necessary for *de novo* synthesis of purine nucleotides. As proliferation of T- and B-cells is largely dependent on the *de novo* pathway, MPA selectively inhibits proliferating lymphocytes [1,2]. MPA has a 4.8-fold higher inhibitory affinity for IMPDH type II than type I [2].

Since its approval by the US FDA in 1995, MMF has proven to be effective in the prevention of acute rejection after renal transplantation. For example, a pooled efficacy analysis showed a decrease of the rate of biopsy-proven acute rejection in renal transplant patients from 40.8% with regimens of ciclosporin, corticosteroids and azathioprine or placebo, to 19.8% and 16.5% with regimens consisting of ciclosporin, corticosteroids and MMF 2 or 3 g/day, respectively [3]. Comparable results have been obtained with MMF in combination with tacrolimus [1]. Strikingly, one recent multi-center study [4] could not find a significant difference in the incidence of acute rejection between MMF and azathioprine in a highly selected population of recipients of a first cadaveric renal transplant. The absence of a positive effect of MMF was explained by an improved exposure to ciclosporin after Neoral® administration compared with previous trials which used Sandimmune®. Nevertheless, MMF has largely replaced azathioprine in most immunosuppressive regimens, considering that 79% of renal transplant recipients in the US used MMF at hospital discharge in 2002 [5]. The success of MMF in solid organ transplantation has also led to its application for prevention of graft-versus-host disease after haematopoietic stem cell transplantation, as well as for treatment of autoimmune disorders such as systemic lupus erythematosus and psoriasis [6]. Another important aspect that may have contributed to its widespread use, is that MMF is considered as easy to use with a fixed dose recommendation of 1 gram twice daily, without the need for monitoring, which is in contrast with the use of ciclosporin, sirolimus or tacrolimus [7].

Despite the successful use of MMF, a growing amount of data suggests that the "one-size-fits-all" strategy for MMF may not be the optimal therapeutic option. With fixed dose therapy a 10-fold between-patient variability in exposure to MPA is observed. In addition, the changing pharmacokinetics of the drug over time, and the large between-patient variability in IMPDH inhibition are arguments that

suggest that individualisation of MMF dose can further improve current MMF practice [1,8,9]. This review focuses on the need for individualisation of MMF therapy and subsequently discusses possible pharmacokinetic and pharmacodynamic methods that could serve as a basis for dose individualisation. For more general aspects about the use of MMF in solid organ transplantation, as well as for detailled information about EC-MPS, the reader is referred to previous papers in this journal [10,11].

3 PHARMACOKINETICS AND PHARMACODYNAMICS OF MYCOPHENOLATE MOFETIL

After oral adminstration, MMF undergoes rapid presystemic de-esterification to MPA [12]. The absorption process of MPA is fast and almost complete, with maximum plasma concentrations generally occurring within 1 h after MMF administration. MPA binds extensively to plasma albumin with a free fraction of < 3% [12]. The protein binding of MPA is presumed to play an important role in the pharmacokinetics of MPA. First, it is the free fraction of MPA that is available for immunosuppressive activity, and, second, MPA is believed to be a restrictively cleared drug. The latter means that it is the free fraction of MPA which is supposed to be available for elimination from the body. The consequence is that an increase of the free fraction of MPA will subsequently result in an increase of MPA clearance [13]. MPA is cleared through metabolism by uridine diphosphate-glucuronosyltransferase (UGT) enzymes in liver, kidney and intestinal mucosa [14,15]. The main metabolite is primarily formed by UGT1A9 and is the phenolic MPA glucuronide (MPAG), which is not pharmacologically active [12,15,16]. MPAG has a protein binding of 82% and is known to displace MPA from its albumin binding sites at high concentrations, which may occur during renal insufficiency [12,17]. Other glucuronide metabolites have been identified: the phenolic glucoside and the acyl glucuronide (AcMPAG) metabolite [15,16,18]. The latter is formed by UGT2B7 and has been shown to be pharmacologically active *in vitro* [1,14]. AcMPAG has been linked to the occurrence of gastrointestinal adverse effects that are frequently observed with MMF therapy, and which are an important reason for MMF dose reductions or for discontinuation of the drug [19]. The pharmacokinetics of MPA are further characterised by an enterohepatic recirculation, in which MPAG is excreted into bile; thereafter MPAG is deglucuronidated in the gut by the intestinal flora to form MPA, which is reabsorped from the colon, leading to a second peak concentration 6 - 12 h after MMF administration [12]. The contribution of enterohepatic recirculation to the total MPA area-under-the-curve (AUC) ranges 10 - 61% in humans [12]. Finally, the glucuronide metabolites are excreted by the kidneys.

The course of inhibition of IMPDH activity runs parallel to the MPA concentration-time profile. Maximal IMPDH inhibition coincides with maximal MPA concentrations ~ 1 h after MMF administration [20,21]. In five dialysis patients, IMPDH inhibition persisted for 4 h, despite MPA concentrations below a mean value of 2.0 mg/L. IMPDH activity returned to baseline 11 h after MMF dosing [22].

EC-MPS was developed to reduce the gastrointestinal adverse effects occurring with the use of MMF. Absorption of MPA following EC-MPS administration is delayed compared with MMF because MPA becomes available for absorption no earlier than in the ileum as a result of the enteric-coating [11]. The result is that maximum concentrations occur 1 - 8 h after EC-MPS intake [23,24]. Despite this difference in pharmacokinetics, several studies have shown bioequivalent MPA exposure after EC-MPS compared with MMF [25,26], and also therapeutic equivalence has been found in stable renal transplant recipients [27].

4 THE NEED FOR MYCOPHENOLATE MOFETIL DOSE INDIVIDUALISATION

The official dose recommendation for MMF in adult renal transplant recipients is to use 2000 mg/day in combination with a calcineurin inhibitor and corticosteroids. This dosing strategy was based on the three so-called pivotal randomised, controlled, double-blind, multicentre trials for MMF [28-30]. These trials included in total 1493 renal transplant recipients and showed that a dose of 3000 mg/day did not significantly reduce the incidence of biopsy-proven acute allograft rejection over a dose of 2000 mg/day, whereas gastrointestinal toxicity and tissue-invasive cytomegalovirus disease occurred significantly more frequently with MMF 3000 mg. Since these studies, data have become available which provide four reasons to question the justification of a fixed MMF dose: 1) the existence of a concentration-effect relationship; 2) large between-patient variability in pharmacokinetics and pharmacodynamics; 3) an increase of MPA exposure over time after transplantation despite a fixed dose; and 4) the influence of co-medication.

4.1 Mycophenolic acid concentration-effect relationship

After a small pilot study in Japanese renal transplant recipients [31], the existence of a strong relationship between exposure to MPA and the risk for acute allograft rejection was demonstrated in a randomised concentration controlled trial (RCCT) of MPA (table 1) [8,32]. A total of 150 renal transplant recipients, who received MMF in combination with ciclosporin were randomised to three AUC target groups: low (16.1 mg*h/L), intermediate (30.3 mg*h/L) and high AUC target group (60.6 mg*h/L). AUC was measured on nine occasions during the first 6 months after transplantation, and the MMF dose was adjusted accordingly. In the low AUC target group, the primary end point of biopsy-proven acute rejection was observed in 27.5% of patients, and in the intermediate and high AUC target groups, corresponding values were 14.9% and 11.5% respectively. Patients experiencing biopsy-proven acute rejection had a significantly lower MPA exposure than patients free from rejection. AUC correlated better than predose level with the incidence of biopsy-proven acute rejection ($p < 0.001$ for AUC versus $p = 0.01$ for predose level). A concentration-effect relationship was also found in paediatric renal transplant recipients [33]. A MPA $AUC_{0-12} < 33.8$ mg*h/L, as well as a MPA predose level < 1.2 mg/L, were associated with a significantly increased risk for acute rejection in the first 3 weeks after renal transplantation ($p = 0.005$ and $p < 0.001$, respectively). Interestingly, a study in 94 adult renal transplant recipients demonstrated that adequate MPA exposure as early as day 3 is associated with a significantly lower incidence of acute rejection during the first 3 months after renal transplantation [34]. A cutoff value for MPA AUC_{0-12} on day 3 of 22 mg*h/L had the best predictive performance for the development of acute rejection.

Although the concentration-effect relationship is well established for ciclosporin cotreated patients, this correlation has not yet been extensively investigated in renal transplant recipients who used tacrolimus as concomitant immunosuppressant drug and a statistically significant relationship has not been found in this patient population (table 1). One study [35] including 100 renal transplant recipients cotreated with tacrolimus, found a trend towards an increased incidence of acute rejection when both tacrolimus AUC_{0-12} was < 150 ng*h/mL and MPA AUC_{0-12} was < 45 mg*h/L ($p = 0.07$).

The relationship between MPA exposure and MMF related adverse events is not as well established as the correlation with the risk for acute rejection. The largest studies including ciclosporin cotreated patients were not able to identify a correlation between MPA exposure and toxicity (table 2) [32-34,36]. The reason may be that it is difficult to distinguish MMF-related toxicity from adverse events resulting from coadministered immunosuppresants, or from other morbidity following transplant surgery.

Table 1 Mycophenolic acid concentration-effect relationship in renal transplant recipients for the risk for acute rejection

Author	n	Subjects	Co-treatment	PK parameter	Result	Ref
Takahashi	32	Adults	CsA	AUC_{0-12}	Higher risk for AR with AUC < 40 mg*h/L	31
Van Gelder	150	Adults	CsA	AUC_{0-12}/ AUC_{0-2} C_0	Higher risk for AR with lower AUC Higher risk for AR with lower C0	32
Pillans	27	Adults	CsA	AUC_{0-6} C_0	Higher risk for AR with AUC < 30 mg*h/L No significant concentration-effect relationship	108
Mourad	31	Adults	CsA, ATG	AUC_{0-12} C_0	Higher risk for AR with lower AUC Higher risk for AR with lower C0	71
Kiberd	94	Adults	CsA, IL2Ab	AUC_{0-12}	AUC on day 3 post-transplant predictive for AR	34
Weber	54	Children	CsA	AUC_{0-12} C_{12} Free AUC_{0-12}	Higher risk for AR with AUC < 34 mg*h/L Higher risk for AR with C0 < 1.2 mg/L No significant concentration-effect relationship	33
Atcheson	42	Adults	CsA (n=32) Tac (n=10) IL2Ab	AUC_{0-6}	No significant concentration-effect relationship	36
				Free AUC_{0-6}	No significant concentration-effect relationship	
Mourad	51	Adults	Tac	AUC_{0-12} C_0	No significant concentration-effect relationship No significant concentration-effect relationship	37
Kuypers	33	Adults	Tac, IL2Ab	AUC_{0-12} Free AUC_{0-12}	No significant concentration-effect relationship No significant concentration-effect relationship	51
Kuypers	100	Adults	Tac, IL2Ab	AUC_{0-12}	No significant concentration-effect relationship	35

In every study, patients also received corticosteroids. AR = Acute rejection; ATG = Anti-thymocyte globuline; C_0 = Predose concentration; C_{12} = Concentration 12 hours after administration; CsA = Ciclosporin; IL2Ab = IL-2 antagonists (basiliximab or daclizumab); MPA = Mycophenolic acid; PK = Pharmacokinetic; Tac = Tacrolimus.

Nevertheless, in tacrolimus cotreated patients, some studies have reported on a significantly higher MPA exposure in patients who experienced adverse effects (leukopaenia, thrombocytopaenia, anaemia or gastrointestinal toxicity) (table 2) [35,37].

Although free MPA is the pharmacologically active moiety [17], no studies have shown a correlation between free MPA levels and the risk for acute rejection (table 1). However, exposure to free MPA was found to correlate with MMF-related toxicity, especially haematological and infectious adverse effects (table 2). This has been shown in both an adult and a paediatric renal transplant population [33,36]. A free MPA AUC_{0-12}-value in the initial phase after transplantation > 0.4 mg*h/L was found to be associated with a significantly higher risk for the presence of leukopaenia and/or infections in the paediatric study [33]. In addition, two case reports found high MPA free fractions and a high free MPA AUC-values in a renal transplant recipient and a pancreas-kidney transplant patient with severe leukopaenia [38,39] (table 2).

In 2001, a consensus meeting provided recommendations with regard to the rational use of MMF in solid organ transplantation [40]. A therapeutic window was proposed of a total MPA AUC range for the early post-transplant phase of 30 to 60 mg*h/L based on the established relationship between

exposure to MPA and the risk for acute rejection in ciclosporin cotreated renal transplant patients [40]. The corresponding window for predose levels were values between 1.0 and 3.5 mg/L. The lower limit of the therapeutic window was established based on the convincing evidence from the afore-described studies. The evidence for the upper limit is less well defined, but the risk for leukopaenia may increase with MPA exposure > 60 mg*h/L.

Table 2 Mycophenolic acid concentration-effect relationship in renal transplant recipients for the risk for adverse events

Author	n	Subjects	Co-treatment	PK parameter	Result	Ref
Van Gelder	150	Adults	CsA	AUC_{0-12}/ $AUC_{0-2/}C_0$	No significant concentration-effect relationship	32
Pillans	27	Adults	CsA	AUC_{0-6} C_0	Higher risk for GI-events with lower AUC No significant concentration-effect relationship	108
Mourad	31	Adults	CsA, ATG	AUC_{0-12} C_0	Higher risk for AE with higher AUC Higher risk for AE with higher C_0	71
Kiberd	94	Adults	CsA, IL2Ab	AUC_{0-12}	No significant concentration-effect relationship	34
Weber	54	Children	CsA	AUC_{0-12} C_0 Free AUC_{0-12} Free C_{max}	No significant concentration-effect relationship No significant concentration-effect relationship Higher risk for AE with free AUC > 0.4 mg*h/L Higher risk for AE with higher C_{max}	33
Atcheson	42	Adults	CsA (n=32) Tac (n=10) IL2Ab	AUC_{0-6} Free AUC_{0-6}	No significant concentration-effect relationship Higher risk for AE with higher free AUC	36
Mourad	51	Adults	Tac	AUC_{0-12} C_0 C_1	Higher risk for AE with higher AUC Higher risk for AE with higher C_0 Higher risk for AE with higher C_1	37
Kuypers	33	Adults	Tac, IL2Ab	AUC_{0-12} Free AUC_{0-12}	No significant concentration-effect relationship No significant concentration-effect relationship	51
Kuypers	100	Adults	Tac, IL2Ab	AUC_{0-12} C_0	Higher risk for AE with higher AUC Higher risk for AE with higher C_0	35
Mudge	1	Adult	CsA, IL2Ab	Free AUC_{0-6} Free fraction	Severe nausea and pancytopaenia with free AUC of 13 mg*h/L and free fraction of 18%	39
Kaplan	1	Adult	CsA	Free AUC_{0-12} Free fraction	Leukopaenia with free AUC of 37 mg*h/L and free fraction of 14%	38

In every study, patients also received corticosteroids. AE = Adverse event; ATG = Anti-thymocyte globuline; C_0 = Predose concentration; C_1 = Concentration 1 hour after administration; CsA = Ciclosporin; GI = Gastrointestinal; IL2Ab =IL-2 antagonists (basiliximab or daclizumab); MPA = Mycophenolic acid; PK = Pharmacokinetic; Tac = Tacrolimus.

4.2 Between-patient variability in mycophenolic acid pharmacokinetics and pharmacodynamics

Besides the concentration-effect relationship, an important reason for questioning the one-dose-fits-all strategy for MMF is the large between-patient variability in MPA exposure. Exposure to MPA varies 10-fold or more between adult patients receiving a kidney, liver or heart transplant after administration of 1000 mg MMF twice daily [1]. For renal transplant recipients, MPA-AUC_{0-12} values were ~ 10 - 100 mg*h/L [13,41]. The between-patient variability in ciclosporin and tacrolimus cotreated renal transplant patients at various moments after transplantation is summarised in table 3. Large variability in MPA pharmacokinetics was confirmed in four population pharmacokinetic analyses [42-45]. In these analyses, reported estimates for between-patient variability in MPA clearance, which determines drug exposure together with dose, ranged from a 2.2-fold to a 5.7-fold variability.

The consequence of the between-patient variability is that standard dose MMF results in MPA AUC_{0-12} values outside the defined target ranges in large proportions of patients [40].

High between-patient variability has also been reported for the pharmacodynamics of MMF, both in IMPDH activity pretransplant [9,22,46] and in MPA-induced IMPDH inhibition post-transplant [20,21,47]. A study in 60 healthy volunteers found IMPDH activity in peripheral blood mononuclear cells (PBMCs) to vary ~ 5.0 and 33 nmol/h/mg protein [46].

Table 3 Between-patient variability in exposure to mycophenolic acid in renal transplant recipients

Author	n	Subjects	Co-treatment	Time after transplantation	PK parameter	Range for MMF 1000 mg twice daily	Ref
Kiberd	94	Adults	CsA, IL2Ab	Week 1	AUC_{0-12}	11.0 - 55.0 mg*h/L	34
Atcheson	32	Adults	CsA, IL2Ab	Day 5	AUC_{0-6}	3.7 - 44.0 mg*h/L	36
Pescovitz	86	Adults	CsA	>6 months	AUC_{0-12} C_0	27.0 - 100 mg*h/L 0.2 - 5.6 mg/L	103
Weber	17	Children	CsA	Week 1	AUC_{0-12} C_0	19.0 – 56.0 mg*h/L 0.5 - 5.2 mg/L	49
				Month 3	AUC_{0-12} C_0	33.0 – 114.0 mg*h/L 1.0 - 2.4 mg/L	
Kuypers	33	Adults	Tac, IL2Ab	Day 7	AUC_{0-12} C_0	17.0 – 71.0 mg*h/L 0.1 - 5.0 mg/L	51
				Month 3	AUC_{0-12} C_0	24.0 – 111.0 mg*h/L 0.5 - 5.7 mg/L	

C_0 = Predose concentration; CsA = Ciclosporin; IL2Ab = IL-2 antagonists (basiliximab or daclizumab); MMF = Mycophenolate mofetil; MPA = Mycophenolic acid; PK = Pharmacokinetic; Tac = Tacrolimus.

4.3 Changes in mycophenolic acid pharmacokinetics and pharmacodynamics over time after transplantation

Exposure to MPA increases with time after transplantation by at least 30 - 50%, despite the use of a fixed MMF dose [1,48-51] or even despite small MMF dose reductions [8]. Mean MPA AUC_{0-12} normalised to MMF 1000 mg, increased from 43 mg*h/L at week 2 to 56 mg*h/L at 3 months after renal transplantation in patients also receiving ciclosporin (n = 17) [50]. In tacrolimus cotreated patients (n = 33), mean MPA AUC_{0-12} increased from 44 mg*h/L at week 1 to 60 mg*h/L at month 3 after renal transplantation [51]. A study analysing the course of MPA AUC_{0-12} over time in patients concomitantly treated with sirolimus (n = 13) did not show an increase over time [50]. An increase in median MPA AUC_{0-12} was also found in 17 children from 41 mg*h/L at week 1 to 65 mg*h/L at month 3 after renal transplantation [49]. Interestingly, one study in ciclosporin-cotreated renal transplant recipients showed that the increase of MPA AUC_{0-12} over time was due to a decrease in MPA clearance, and only occurred in patients with impaired renal function [13]. Patients with impaired renal function (defined as the need for dialysis in the first week post-transplant or a serum creatinine > 4 mg/dL, n = 13) had a mean MPA-AUC_{0-12} of 21 mg*h/L at day 4, which increased to 36 mg*h/L by day 28 post-transplant. However, patients without initial renal impairment (n = 20) had a mean AUC_{0-12} of 40 mg*h/L both on day 4 and on day 28. This suggests that recovering renal function accounts for the time-dependent changes of MPA pharmacokinetics. The proposed mechanism is that when renal function improves over time post-transplant, MPA protein binding increases as a result of less displacement by MPAG [13,17]. The resulting decrease in MPA free fraction in patients with improving renal function (5.9% at day 4 and 3.0% at day 28 after transplantation) causes a decrease in clearance, as MPA is supposed to be restrictively cleared [13]. Renal function was quite stable in the study combining MMF with sirolimus, and this may explain the absence of an increase of MPA exposure [50]. Other factors, such as plasma albumin levels or dose changes of concomitant immunosuppressive drugs, which change over time following transplantation, may contribute to the increase of MPA exposure as well [50,52].

One study has reported time-dependent changes in the pharmacodynamics of MMF [53]. Renal transplant recipients using MMF for > 2 years (n = 8) had significantly less inhibition of IMPDH than patients who used the drug for < 1 year (n = 9), suggesting induction of IMPDH after long-term treatment with MMF. An important caveat in this study was the fact that IMPDH activity was measured in whole blood. The pharmacological effect of MMF solely arises from IMPDH inhibition in PBMCs, but IMPDH is also present in erythrocytes. It has been shown that IMPDH is induced during the first 3 months after transplantation, but only in erythrocytes and not in PBMCs [54,55]. Therefore, PBMCs are considered the relevant matrix for studies into IMPDH inhibition after MMF administration.

4.4 The influence of co-medication on mycophenolic acid pharmacokinetics

Several studies showed that different combinations of drugs in immunosuppressive regimens result in differences in MPA exposure [56-60]. A study in stable renal transplant recipients found that patients on MMF and tacrolimus therapy (n = 18) had significantly higher mean MPA AUC_{0-12} values compared with patients on MMF and ciclosporin therapy (n = 7) (50 versus 32 mg*h/L, respectively; $p < 0.02$) [56]. MPAG AUC_{0-12}-values were significantly lower in the tacrolimus group. This difference in MPA exposure between patients cotreated with ciclosporin or tacrolimus was confirmed by several other groups [36,44,58]. At first, inhibition of the glucuronidation of MPA to MPAG by tacrolimus was thought to increase MPA exposure, which was supported by *in vitro* data [56]. However, these findings are also consistent with the hypothesis that concomitant use of ciclosporin decreases MPA exposure. Evidence for this was provided by a study in 19 renal transplant recipients, demonstrating significantly increasing

median MPA predose levels when ciclosporin was withdrawn 6 months after renal transplantation while MMF dose was fixed [59]. An animal study revealed that ciclosporin, but not tacrolimus, had a significant impact on MPA AUC_{0-24}, probably as a result of ciclosporin induced interruption of the enterohepatic recirculation of MPA [61]. Supportive clinical evidence for the inhibitory effect of ciclosporin on the enterohepatic recirculation of MPA was provided by a population pharmacokinetic study, which showed that total MPAG clearance was lower in ciclosporin cotreated patients than in tacrolimus cotreated patients, despite a similar renal function in both groups [62]. The drug-interaction between ciclosporin and MPA is now well documented [63], and it is likely that this mechanism also contributed to the initial observations [56]. The effect of ciclosporin on MPA exposure probably also explains the observed higher MPA AUC_{0-12} in patients cotreated with sirolimus compared to patient cotreated with ciclosporin [50,64].

Although a study in seven healthy volunteers suggested that MPA absorption was significantly decreased when MMF was concomitantly administered with iron preparations [65], two studies in renal transplant patients, one in the initial phase [66] and one in the maintenance phase after transplantation [67], could not confirm an interaction between these two drugs. Antacids do interact with MMF, forming non-absorbable complexes, resulting in a decreased bioavailability [12]. Cholestyramine is known to interfere with the enterohepatic recirculation of MPA once MPAG has been excreted in the gut [12]. Recently, a case-report presented a drug-interaction between MMF and rifampin in a heart-lung transplant patient, in which rifampin caused a 2-fold lower MPA $AUC_{0-12,}$ probably as a result of induction of UGT or MRP2 [68].

Finally, significantly lower mean MPA AUC_{0-12} was demonstrated in 26 patients at 6 months after renal transplantation, with MMF 1000 mg in the presence of glucocorticosteroids (51 mg*h/L), compared with steroid tapering at 9 months (55 mg*h/L) or compared with steroid withdrawal at 21 months (67 mg*h/L) [52]. An explanation for these results may be enhanced UGT activity caused by exposure to steroids as has been shown for other drugs [69].

5 HOW TO PERFORM MYCOPHENOLATE MOFETIL DOSE INDIVIDUALISATION?

With the need for individualisation of MMF therapy, the question arises how to individualise the MMF dose. Two, not necessarily mutually exclusive, approaches for dose individualisation may be taken into consideration. The first is based on monitoring therapy, either on a pharmacokinetic or on a pharmacodynamic basis, and the second is based on demographic factors explaining between-patient variability in MPA exposure.

5.1 Monitoring mycophenolate mofetil therapy: a pharmacokinetic approach

By increasing the MMF dose in individuals with low MPA exposure and decreasing the dose in those with high MPA levels between-patient variability in exposure can be reduced, thus optimising exposure to MPA compared to a fixed dose MMF dosing regimen. This can potentially increase overall efficacy and reduce drug-related toxicity. At present, three studies are ongoing that investigate whether a concentration-controlled MMF dosing regimen results in less acute rejection and reduced toxicity compared to a fixed dose MMF dosing regimen: the fixed dose versus concentration controlled (FDCC) study, the OptiCept study in the US and the Apomygres study in France. Interim results from

the Apomygres study after 6 months follow-up showed that there was a trend in the concentration-controlled group (n = 70) towards less biopsy-proven acute rejections compared with the fixed dose group (n = 67) (5 versus 13 episodes, respectively, p = 0.054) [70]. No significant differences were observed between the two groups in MMF-related toxicity. These preliminary results are promising and the results of the larger FDCC and OptiCept studies are eagerly awaited.

Besides effective, therapeutic drug monitoring (TDM) also needs to be feasible. Large within-patient variability causes the need for frequent concentration measurements to keep exposure on target, which makes TDM inefficient and possibly unfeasible. Several studies reported large variability within patients over time for MPA exposure, with CV-values of 40% - 50% for the initial period after renal transplantation and ≤ 30% for the period thereafter [8,51]. The variability for the initial period may have been overestimated as it is unclear from these studies whether the CV-values for (random) within-patient variability were corrected for the structural increase of MPA AUC_{0-12} over time.

Another aspect regarding the feasibility of TDM of MPA is which parameter is the most suitable to obtain a reliable estimate of MPA exposure. MPA exposure is best reflected by a full MPA AUC_{0-12}, and AUC_{0-12} also has the best correlation with the risk for acute rejection [8,32]. However, before a full AUC_{0-12} can be calculated, at least eight MPA concentration-time samples need to be drawn covering the total 12-h dosing interval. This is costly, time-consuming, inconvenient for the patient and not feasible in an out-patient clinic setting. MPA predose levels have a weaker correlation with the risk for acute rejection, but have the advantage of being convenient [32,71]. Higher within-patient variability of predose levels, compared with full AUC_{0-12} in the first weeks after transplantation may pose a drawback [13]. Moreover, the frequently observed weak correlation between predose levels and AUC_{0-12} suggests that predose levels do not always reflect exposure adequately [72-74]. A suitable alternative may be estimation of full MPA AUC_{0-12} by a limited sampling strategy. Several limited sampling strategies have been proposed for MPA based on multiple regression analyses, and mostly consisted of 3 or 4 samples drawn during the first 2 - 6 h of a dosing interval, with correlation coefficients ≤ 0.95 [72-76]. The advantage is a reliable estimation of MPA exposure, from no more than 2- to 6-h stay. A disadvantage is that MPA plasma samples need to be drawn at exact time-points after MMF dosing. This is not always possible in clinical practice and may lead to biased AUC_{0-12} estimates. A sophisticated solution to this problem is the use of a maximum *a posteriori* Bayesian estimation of the MPA AUC_{0-12} [201]. This method also uses a limited sampling strategy, but estimates AUC by combining the concentration measurements with information from a population pharmacokinetic model for MPA. An important advantage is more flexibility regarding the timing of sample drawing [77]. A disadvantage may be that the development of a Bayesian algorithm for exposure estimation is labour-intensive and statistically complex. Two of such Bayesian algorithms for estimation of MPA exposure in ciclosporin cotreated patients have been published [43,77].

It is important to note that despite the bioequivalence regarding MPA exposure after EC-MPS compared with MMF, concentration-time curves of MPA after oral administration of EC-MPS often exhibit complex and highly variable absorption profiles, with delayed peak concentrations occuring after more > 3 h [23]. This may have implications for TDM of MPA with the use of EC-MPS. Limited sampling strategies may not reflect full MPA AUC_{0-12} adequately, and may, therefore, be unsuitable. The same might be true for predose levels [11]. Consequently, the above described principles for TDM of MPA can not be uncritically extrapolated to EC-MPS. Specific studies are necessary for EC-MPS to assess the usefulness and feasibility of TDM of MPA, and to determine which surrogate parameter adequately reflects MPA exposure.

5.2 Monitoring mycophenolate mofetil therapy: a pharmacodynamic approach

A new and promising approach for MMF dose individualisation is pharmacodynamic monitoring. Measurement of a biomarker is the next best thing over measuring clinical end points directly, as biomarkers are supposed to correlate more closely with efficay than drug concentrations, and are thought to better reflect the variability in both pharmacokinetics and pharmacodynamics [78]. Inhibition of IMPDH activity may, in theory, prove to be a suitable biomarker for pharmacodynamic monitoring of MMF therapy. A relationship between pretransplant IMPDH activity and clinical outcome has been found [9]. Lower pretransplant IMPDH activity was associated with an increased incidence of MMF dose reductions, which is a surrogate end point for adverse events. Furthermore, high pretransplant IMPDH activity (above the cutoff value of 8.5 nmol/mg protein/h) was associated with a higher risk of acute rejection. These findings suggest that measurement of IMPDH activity may be suitable to monitor clinical efficay and toxicity of MMF therapy. However, before dose adjustments can be recommended based on IMPDH activity, more information is necessary about the relationship between MMF dose and IMPDH activity, about the magnitude of intraindividual variability in IMPDH activity and about the relationship between clinical outcomes and post-transplant IMPDH activity [79]. A disadvantage of pharmacodymanic monitoring is the technically complex and time-consuming measurement of IMPDH activity, generally consisting of isolation of PBMCs and a high-performance liquid chromatography (HPLC) method [22,46,80].

5.3 Demographic factors as basis for mycophenolate mofetil dose individualisation

Another possibility for mycophenolate mofetil dose individualisation is to rely on patient characteristics that explain an important part of the between-patient variability in MPA exposure. The following characteristics may be considered: comedication, factors affecting MPA protein binding, (pharmaco)genetics, race and time after transplantation. It needs to be emphasised that, in theory, MMF dosing based on these factors can optimise MPA exposure, but this needs to be confirmed in prospective trials.

5.3.1 The impact of comedication on dose individualisation

As discussed in section 4.4, MPA exposure is significantly lower when used in combination with ciclosporin, compared with tacrolimus or sirolimus [36,44,50,52,59,60,62]. In ~ 50% of the patients, exposure was below the lower limit of the therapeutic window (30 mg*h/L) in the first weeks after renal transplantation when MMF 1000 mg twice daily was combined with ciclosporin [36,44,50]. As a result, these patients are at increased risk for developing acute rejection in the first weeks after transplantation [34]. Therefore, outcome in patients cotreated with ciclosporin may be improved with higher MMF doses than the currently recommended MMF 1000 mg twice daily in the immediate post-transplant phase. By subsequently measuring MPA concentrations, dose could then be adjusted further.

5.3.2 The impact of renal function and plasma albumin levels on dose individualisation

As already mentioned under 3 and 4.3, MPA protein binding is an important determinant of total MPA exposure, and appears to be influenced by renal function. A study found an effect of impaired renal function on total MPA exposure, but could not show a change in MPA free fraction, because free MPA levels were not measured [74]. Other studies did measure free MPA and showed elevated free MPA concentrations in situations of impaired renal function [13,17,51,81,82]. However, a subsequent decrease of total MPA exposure could not always be found in these studies [51,81,82]. Besides renal

function, MPA protein binding also seems to be influenced by plasma albumin levels. As expected on basis of the restrictive clearance concept, low albumin levels were associated with low total MPA exposure [44]. Again, the presence or absence of an effect on free MPA could not be shown because free MPA levels were not measured [44]. Other studies did find increased free MPA concentrations in patients with low albumin levels [82,83], but found total MPA to be unaltered [82].

Although renal function and plasma albumin levels can partly explain differences between patients in exposure to both free and total MPA, the conflicting results obtained from different studies clearly illustrate that the influence of these factors may be overshadowed by other effects. Therefore, it is currently unknown to what extent MMF dose adjustments are indicated in the presence of poor renal function or hypoalbuminaemia.

5.3.3 The impact of pharmacogenetics on dose individualisation

It is estimated that 20 - 100% of interindividual variability in drug disposition and effects can be attributed to genetic differences between individuals [84-87]. The possibility that, in addition to variables such as renal function, plasma albumin level and comedication, an individual's response to MPA treatment is also genetically determined has, therefore, been considered [88]. Pharmacogenetic research with regard to MPA has focused on the following enzymes: UGT, MRP2 and the IMPDH type I and II.

In adults, there exist marked interindividual differences in UGT expression and glucuronidation activity [89]. For example, a > 9.5-fold difference in MPA glucuronidation activity was found in hepatic microsomes, which correlated with UGT1A9 protein levels [90]. Evidence for a genetic basis of the variable UGT expression was provided recently with the identification of several single-nucleotide polymorphisms (SNPs) in the *UGT1A1*, *UGT1A7* and *UGT2B7* genes. Some of these SNPs result in the complete or partial loss of glucuronidation activity [89,91,92]. Preliminary results from a study among 33 kidney transplant recipients have indicated that the -840G > A SNP in *UGT2B7* may be associated with an increased production of the pharmacologically active, and possibly toxic, AcMPAG metabolite [19,93]. In addition, SNPs have been discovered in the coding and promoter region of the *UGT1A9* gene, which is considered to be the UGT isozyme most important for MPA glucuronidation [90,91,94]. Of all *UGT1A9* promoter SNPs investigated, the -2152C > T and -275T > A SNPs were found to have the strongest association with hepatic UGT1A9 protein content [90]. Carriers of these closely-linked SNPs had roughly 2-fold higher UGT1A9 protein levels compared with carriers of the wild-type promoter and with noncarriers of the -2152C > T/-275T > A SNPs. Importantly, *in vitro* MPA glucuronidation activity was 2.1-fold higher in -2152C > T/-275T > A carriers [90]. Recently, it was demonstrated that the -2152C > T and -275T > A SNPs in *UGT1A9* are associated with significantly lower MPA exposure in the early phase after renal transplantation [95]. The less frequently occurring *UGT1A9*3* SNP was associated with a higher MPA exposure, which is in agreement with the previously described reduction of *in vitro* enzymatic activity [90,91,95]. These observations demonstrated for the first time that *in vivo* interindividual variability in the pharmacokinetics of MPA can be partially explained by genetic variation. Given the high allelic frequency of the *UGT1A9* -2152C > T and -275T > A SNPs (~ 15% in Caucasians), and the two-fold reduction in MPA exposure in comparison with non-carriers, these findings are also likely to be clinically relevant and offer both a rationale and a means for MMF dose individualisation.

Several mutations and SNPs have been identified in the gene encoding for MRP2 [96-99]. Some of these genetic alterations lead to the complete loss of MRP2 transport activity and cause the human Dubin-Johnson syndrome [96]. This syndrome is a rare, autosomal recessive disorder characterised by a deficiency in the transport of the MRP2 substrates monoglucuronosyl and bisglucuronosyl bilirubin into bile, which leads to a chronic conjugated hyperbilirubinemia and pigment deposition in the liver [100].

Only preliminary results regarding the effects of *MRP2* SNPs on the pharmacokinetics of MPA have been published [93]. Among 14 renal transplant recipients treated with MMF plus sirolimus, the MPAG to MPA AUC_{0-9} was smaller in carriers of the *MRP2* -24C > T promoter SNP compared with wild-type patients [93]. This finding suggests that the -24C > T SNP leads to an increased secretion of MPAG into bile. This effect was not apparent as a likely result of the inhibitory effect of ciclosporin on MRP2 in 19 patients who received ciclosporin instead of sirolimus [93].

Several SNPs have been identified in exon 10 of the IMPDH type I gene [101], but their effects on IMPDH expression and activity, or their relationship with the response to MMF therapy, are unknown. However, it is possible that SNPs in the *IMPDH* genes form an explanation for the marked interindividual variation in IMPDH activity, and may even predict transplantation outcome in patients treated with MMF [9].

Future research will have to show whether SNPs in *UGT*, *MRP2* and *IMPDH* genes form a suitable tool for MMF dose individualisation.

5.3.4 The impact of race on dose individualisation

From a *post-hoc* analysis of the three pivotal trials of MMF [28-30], African-American patients were found to benefit from MMF 1500 mg twice daily, instead of the standard dose of MMF 1000 mg twice daily [102]. From this analysis it was concluded that African-American patients need, at least in the first 6 months after transplantation, MMF 1500 mg twice daily to obtain the same acute rejection incidence as non-black patients. Two studies, one during the maintenance period and one during the initial period after renal transplantation, investigated whether the higher dose requirement was caused by altered MPA pharmacokinetics in African-Americans compared to Caucasians. No significant differences in pharmacokinetics (clearance and AUC_{0-12}) were found between the two ethnic populations [13,103]. The higher MMF dose, and thus exposure, requirement is likely to have an immunologic origin. A large analysis of the US Renal Data Registry, including 57926 patients, found that African-American renal transplant recipients had a 33% lower risk for death with a functioning graft with MMF, compared with azathioprine. This risk reduction was comparable with the risk reduction in Caucasian patients of 41% [104]. Unfortunately, this study did not document whether this result has been reached with comparable MMF doses in both racial groups.

5.3.5 The impact of the increase of MPA exposure over time post-transplant on dose individualisation

As discussed in section 4.3, the increase in MPA exposure over time is likely to be caused by improving renal function, changes in the exposure to concomitantly administered immunosuppressive agents, especially ciclosporin and corticostroids, and other factors changing over time after transplantation. As a result of the gradual increase in MPA exposure, a subset of patients will exceed the target window if on standard dose of MMF 1000 mg twice daily 6 - 12 months after transplantation. This is most likely in patients who are not cotreated with ciclosporin and who have good renal function [13,50]. It is too early to recommend MMF dose reductions in these patients after ≥ 6 months post-transplant. The recommended target window [40] is based on a combination of MMF with a calcineurin inhibitor during the first 6 months after renal transplantation, and other target values may apply for other combinations and for the period after the first 6 months. Besides, the increased MPA exposure may be very welcome in non-calcineurin inhibitor-containing regimens, or may allow dose reductions of calcineurin inhibitor therapy. Therefore, in patients who tolerate such levels well, dose reductions may not be necessary.

6 EXPERT OPINION

MMF has been a very succesful immunosuppressive agent in renal transplantation over the past 10 years. Acute rejection rates are lower, the incidence of late acute rejection episodes has been decreased and a beneficial effect on the long-term outcomes has been demonstrated compared with azathioprine [105-107]. This all has been achieved with a fixed dose MMF regimen. However, this one-size-fits-all dosing regimen leads to suboptimal MPA levels in subgroups of renal transplant patients, possibly leading to unfavourable outcomes. Optimisation of MPA exposure through dose individualisation may further improve the use of MMF in these patients. For instance, patients who also receive ciclosporin may benefit from higher MMF doses than currently recommended immediately after transplantation to reach target exposure as early as possible. Also renal transplant recipients with impaired renal function or low plasma albumin may have suboptimal MPA exposure with standard dose therapy. However, before mycophenolate mofetil dose adjustments can be recommeneded when renal function is impaired or plasma albumin level is low, more information is necessary about the free MPA exposure, which may be unaltered or elevated in these situations. Furthermore, therapeutic drug monitoring may be suitable to select those patient with altered total MPA exposure, but definitive proof has to be awaited. This proof is likely to come from the three studies investigating the efficacy of therapeutic drug monitoring of MPA: OptiCept, FDCC, and Apomygres.

With regard to the succes of the fixed dose MMF regimen, it would also be interesting to perform a pharmacoeconomic evaluation to compare the costs and the benefits of a pharmacokinetically-based MMF dosing regimen with the current practice.

IMPDH monitoring is a new and promising approach for MMF dose individualisation, as this biomarker may be more closely related to clinical outcome than MPA concentrations. The difficult measurement of IMPDH activity compared with the determination MPA plasma concentrations poses a drawback for application in daily routine practice. A study comparing MMF dosing based on IMPDH monitoring with pharmacokinetically guided dosing, could elucidate if, and under which circumstances, IMPDH activity measurement has additional value over measuring MPA exposure. The identification of patients with extremely high or low pretransplant IMPDH activity may provide a valuable tool in the future for dose individualisation, or to select patients who are not the most suitable candidates for MMF therapy [9].

Importantly, MMF is increasingly being used for other indications than the prevention of acute rejection in solid organ transplantation, for instance the prevention of graft-versus-host disease after stem cell transplantation, and the treatment of autoimmune diseases. The principles of large variability, influence of co-medication and the existence of a concentration-effect relationship may also apply to these indications. Therefore, for optimal treatment, studies are not only necessary to investigate the efficacy of MMF, but also to elucidate the need and ways of dose individualisation in indications outside the field of solid organ transplantation.

Individualisation of MMF dose based on free MPA concentrations may be of value in situations where total MPA exposure does not adequately reflect free MPA exposure, for instance when albumin levels are low, or when renal function is impaired. However, data on the relationship between free MPA concentrations and the risk for acute rejection are lacking and the difficult measurement of free MPA may be a limitation.

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Chapter 1.2

Aims and Scope

In science, knowledge based on experience is used to develop an idea or theory. Predictions about future events that are based on that idea or theory are tested in a scientific experiment to obtain new experiences. The newly generated experiences, or observations, are compared with the predictions. If the new experiences match with the predictions, the idea or theory is confirmed. If not, the idea or theory is falsified [1-3].

The experience with mycophenolate mofetil encompasses several observations that illustrate the need for individualisation of mycophenolate mofetil therapy: 1) the risk for acute rejection is lower when mycophenolic acid (MPA) exposure is higher, 2) MPA exposure is subject to a 10-fold between-patient variability, 3) MPA exposure changes over time after transplantation despite a fixed mycophenolate mofetil dose, and 4) MPA exposure is influenced by the use of co-medication. This experience can also be used to develop ideas about when and how to individualise the mycophenolate mofetil dose. The specific predictions, or hypotheses, reflecting these ideas, and that will be tested in this thesis, are:

1. Demographic factors that contribute to the variability in the pharmacokinetics of MPA may serve as a rationale for mycophenolate mofetil dose individualisation. On the basis of the literature described in chapter 1.1, demographic factors which are likely to be suitable are:

- **a** Co-medication, especially cyclosporine as concurrently used immunosuppressive drug
- **b** Factors affecting MPA protein binding
- **c** Time after transplantation

Based on general principles, further demographic factors may be:

- **d** Co-morbidity
- **e** The type of transplant.

For demographic factors that have a significant contribution to the variability in MPA pharmacokinetics, the underlying mechanism will be investigated.

2. Therapeutic drug monitoring of MPA provides a suitable tool for mycophenolate mofetil dose individualisation with regard to:

- **a** Reducing variability between patients in exposure to MPA, resulting in increasing the proportion of patients reaching target concentrations
- **b** When, how often, and which MPA exposure parameter to measure, to obtain optimal MPA exposure.

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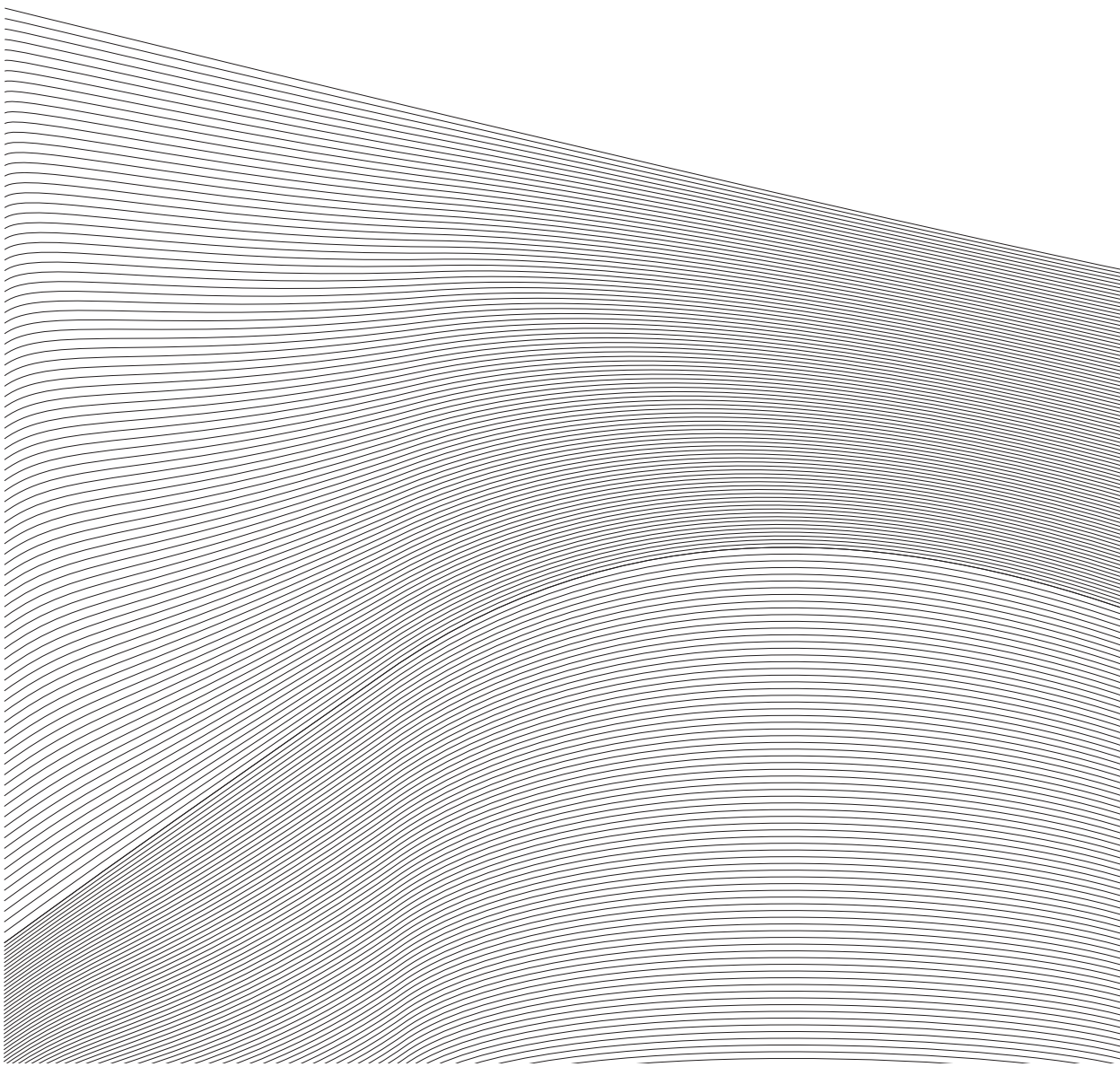
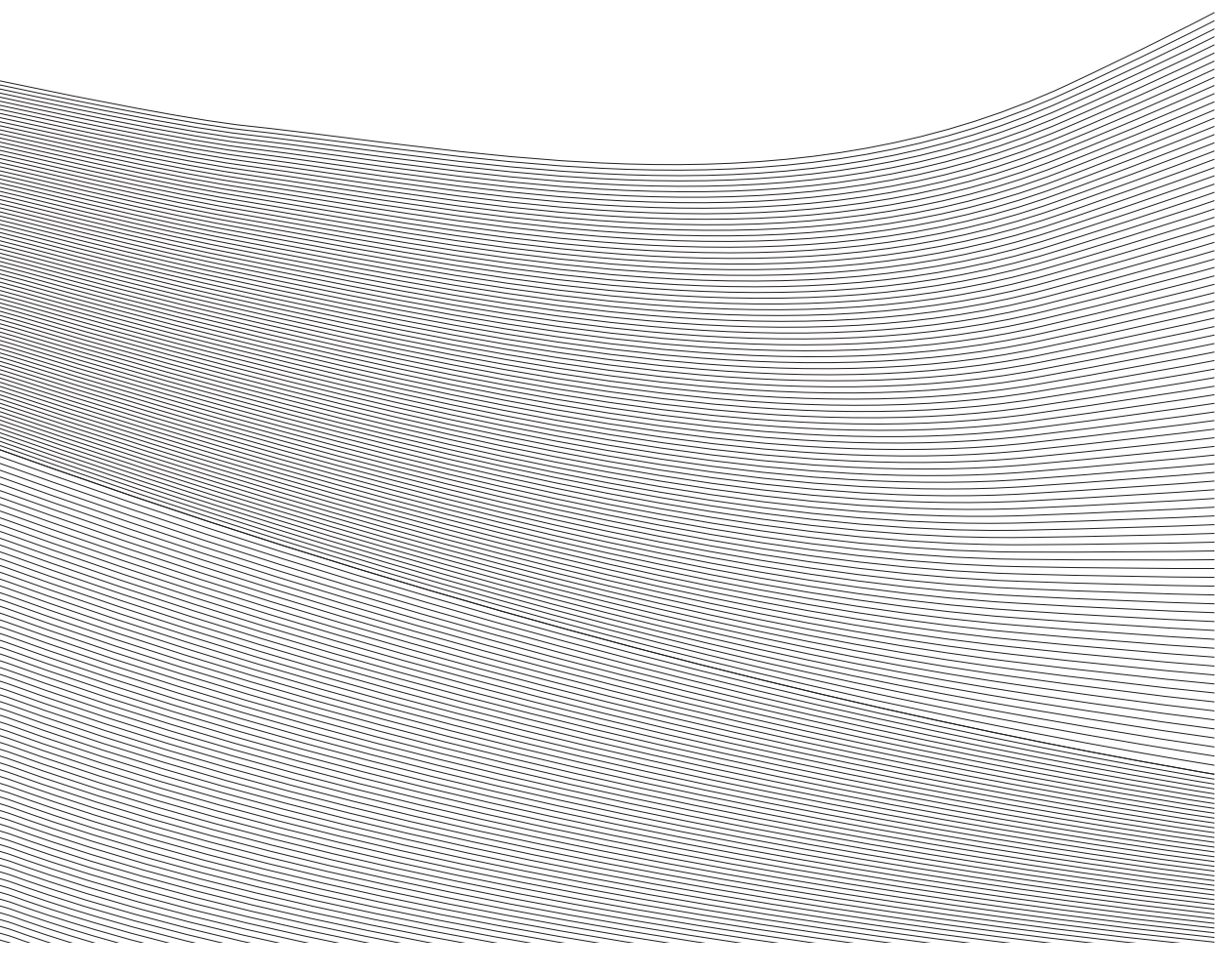


Chapter 2

EXPLAINING VARIABILITY IN MYCOPHENOLIC ACID PHARMACOKINETICS



Chapter 2.1

Mycophenolic acid population pharmacokinetics in renal transplant recipients

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ABSTRACT

Background: Mycophenolate mofetil is the prodrug of mycophenolic acid (MPA) and is used as an immunosuppressant following renal, heart, lung and liver transplantation. Although MPA plasma concentrations have been shown to correlate with clinical outcome, there is considerable pharmacokinetic variability. Consequently, it is important to study demographic and pathophysiological factors that may explain pharmacokinetic variability within and between patients.

Objective: The aim of the study was to develop a population pharmacokinetic model for MPA following oral administration of mycophenolate mofetil, and evaluate relationships between patient factors and pharmacokinetic parameters.

Patients and methods: Pharmacokinetic data were obtained from a randomised concentration controlled trial involving 140 renal transplant patients. Pharmacokinetic profiles were assessed on nine occasions during a 24-week period. Plasma samples for description of full 12-hour concentration-time profiles on the first three sampling days were taken predose, 0.33, 0.66, 1.25, 2, 6, 8 and 12 hours after oral intake of mycophenolate mofetil. For the remaining six occasions, serial plasma samples were taken according to a limited sampling strategy pre-dose and at 0.33, 0.66, 1.25, and 2 hours after mycophenolate mofetil administration. The resulting 6523 plasma concentration-time data were analysed using non-linear mixed effects modelling (NONMEM).

Results: The pharmacokinetics of MPA were best described by a two-compartment model with time-lagged first-order absorption. The following population parameters were estimated: absorption rate constant (k_a) 4.1 h^{-1}, central volume of distribution (V1) 91 L, peripheral volume of distribution (V2) 237 L, clearance (CL) 33 L/h, intercompartment clearance (Q) 35 L/h and absorption lag time 0.21 h. The between-patient variability for k_a, V1, V2 and CL was 111%, 91%, 102% and 31%, respectively; estimates for within-patient variability for k_a, V1 and CL: 116%, 53% and 20% respectively. For MPA clearance statistically significant correlations were found with creatinine clearance, plasma albumin concentration, gender and daily cyclosporin dose ($p<0.001$). For V1, significant correlations were identified with creatinine clearance and plasma albumin concentration ($p<0.001$).

Conclusions: The developed population pharmacokinetic model adequately describes the pharmacokinetics of MPA in renal transplant recipients. The identified correlations appear to explain part of the observed between- and within-patient pharmacokinetic variability. The clinical consequences of the observed correlations remain to be investigated.

INTRODUCTION

Mycophenolic acid (MPA) is the active component of the prodrug mycophenolate mofetil (CellCept®, Roche Laboratories, Nutley, NJ, USA) and is used to prevent acute rejection in lung, heart, liver and renal transplantation [1]. MPA decreases *de novo* purine biosynthesis by inhibiting inosine monophosphate dehydrogenase reversibly and noncompetitively. As a result the proliferation of T and B lymphocytes is suppressed [2]. The pharmacokinetics of MPA after oral administration of a mycophenolate mofetil dose are described by presystemic de-esterfication to MPA during a rapid and almost complete absorption phase [3]. MPA reached maximum plasma concentrations (C_{max}) within 1 hour in most renal transplant recipients [4,5], and is extensively bound to albumin with an average protein binding of 97.5% [6,7]. MPA is mainly metabolised in the liver by uridine diphosphate- glucuronosyltransferase to the inactive 7-O mycophenolic acid glucuronide (MPAG) [4].

MPA exhibits considerable pharmacokinetic variability between and within patients. Its pharmacokinetics are complex since pharmacokinetic parameters change over time following transplantation and appear to be influenced by renal function, albumin levels and drug interactions [8]. Interestingly, a randomised concentration controlled trial (RCCT) [9,10] led to the suggestion that the incidence of acute rejection and adverse effects is minimised when MPA exposure (area under the plasma concentration-time curve [AUC]) lies between values of 30 and 60 mg*h/L [11]. Consequently, therapeutic drug monitoring (TDM) may be useful in reducing and minimising inter-individual variability in MPA exposure [9,11,12]. At present, TDM is not applied on a routine basis, but studies assessing the beneficial effects of TDM in mycophenolate mofetil therapy are currently ongoing.

In this study, a clinically applicable population pharmacokinetic model was developed for MPA to elucidate its complex pharmacokinetics. Between- and within-patient pharmacokinetic variability was quantified and relationships between pharmacokinetic parameters and patient factors were investigated. Clearly, knowledge derived from the population pharmacokinetics of MPA could improve effective administration of mycophenolate mofetil and may be used to more efficiently apply TDM.

PATIENTS AND METHODS

Patients

Concentration-time data from 140 renal transplant patients who participated in the RCCT were analysed retrospectively. A detailed description of the methods of the RCCT was published previously [9,10]. Briefly, its goal was to investigate the relationship between exposure to MPA (AUC, minimum concentration [C_{min}], C_{max}) and outcome (incidence of acute rejection, adverse effects). Patients aged 18 year or older were randomly assigned to three MPA AUC target groups: low (AUC 16.1 mg*h/L), intermediate (AUC 32.2 mg*h/L) or high (AUC 60.6 mg*h/L). Starting mycophenolate mofetil doses were 450 mg twice daily for the low AUC target group, 950 twice daily for the intermediate AUC target group and 1700 mg twice daily for the high AUC target group. All patients received cyclosporin and corticosteroids as concomitant immunosuppressive therapy according to routine practice.

Sampling Procedure

Concentration-time samples were collected on nine occasions, on days 3, 7, 11, 21, 28, 56, 84, 112 and 140 after transplantation. Plasma samples for description of full 12-hour concentration-time profiles on the first three sampling days were taken pre-dose, 0.33, 0.66, 1.25, 2, 6, 8 and 12 hours after oral intake of mycophenolate mofetil. For the remaining six occasions, serial plasma samples were taken according to a limited sampling strategy pre-dose and at 0.33, 0.66, 1.25, and 2 hours after mycophenolate mofetil administration [10]. Mycophenolate mofetil dosages were adjusted according to the MPA AUC assessments, targeting at the afore-mentioned AUC values for the three groups. Patients were required to fast overnight prior to dose administration and for the first 2 hours of the profile after taking the medication. MPA was measured using a validated high-performance liquid chromatography method described elsewhere [13]. The lower limit of quantification was 0.1 mg/L. On each sampling day, routine laboratory tests were performed. Graft function was closely monitored; delayed graft function (DGF) was defined as the need for dialysis in the first week after transplantation. Patient characteristics and biochemical parameters are summarised in table 1.

Table 1 Patient demographics and biochemical parameters

Characteristics		**Median (range)**		
		Day 3	**Day 28**	**Day 140**
AUC target group				
Low (16.1 mg*h/L)	n=	47	45	41
Intermediate (32.2 mg*h/L)	n=	45	41	33
High (60.6 mg*h/L)	n=	48	39	25
Gender				
Male	n=	88	79	64
Female	n=	52	46	35
Race				
Caucasian	n=	131	117	93
Black	n=	3	2	1
Other	n=	6	6	5
Diabetes Mellitus	n=	7	6	5
DGF	n=	23	-	-
Age (years)		50 (19-70)	51 (19-70)	50 (19-69)
Body weight (kg)		69 (37-104)	66 (38-103)	70 (44-98)
Creatinine (µmol/L)		211 (62-1232)	132 (62-906)	123 (79-255)
Creatinine clearance (mL/min)		31 (4-142)	50 (7-110)	60 (25-123)
Albumin (g/L)		34 (22-45)	37 (23-50)	40 (32-53)
SGPT (U/L)		15 (2-653)	13 (3-89)	11 (3-54)
SGOT (U/L)		18 (3-289)	12 (3-38)	12 (2-10)
Hemoglobin (g/dL)		9.7 (6.7-13.2)	10.3 (6.8-14.8)	11.8 (8.9-18.8)
Cyclosporine daily dose (mg)		600 (0-1400)	450 (200-1400)	300 (150-700)
Patients not using cyclosporine	n=	3	0	0
MMF dose (mg bid)				
Low AUC target group (16.1 mg*h/L)		450 (450-450)	550 (350-850)	450 (250-950)
Intermediate AUC target group (32.2 mg*h/L)		950 (950-950)	1300 (500-1750)	1050 (400-1950)
High AUC target group (60.6 mg*h/L)		1700 (1700-1700)	2200 (1100-2200)	2100 (1200-2200)

MMF = mycophenolate mofetil, DGF = delayed graft function, AUC = area-under-the-curve. Normal values for creatinine: 65-115 µmol/L for males and 55-90 µmol/L for females, for albumin: 35-50 g/L, for SGOT: < 37 U/L for males and < 31 U/L for females, for SGPT: < 41 U/L for males and < 31 U/L for females, for hemoglobin: 13.8-16.9 mmol Fe/L for males and 12.1-15.3 mmol Fe/L for females

Pharmacokinetics

All data were analysed simultaneously using the nonlinear mixed-effects modelling software program (NONMEM Version V, level 1.1) [GloboMax LLC, Hanover, MD, USA]. Because NONMEM estimated pharmacokinetic parameters for MPA, mycophenolate mofetil doses were converted to the equivalent MPA content by multiplying the mycophenolate mofetil dose by 0.739. A logarithmic transformation (natural logarithm) of the concentration-time data was performed because random effects were not sufficiently distributed around zero without the transformation. The first order estimation method was used throughout the entire model building process, due to the computational intensity of the model using the first order conditional estimation method.

Several structural models were tested. Models with one, two or three compartments were evaluated, as well as models with and without lag time. Furthermore, it was evaluated whether absorption was best described as a first- or zero-order process. Pharmacokinetic parameters were estimated in terms of clearance (CL), central and peripheral volume of distribution (V1, V2, V3) and intercompartment clearances (Q2, Q3) (NONMEM code TRANS4). Since bioavailability (F) could not be quantified, CL, Q and V values of MPA correspond to the ratios CL/F, Q/F and V/F, respectively. Between-patient variability for all pharmacokinetic parameters was modelled using an exponential error model. For example, MPA clearance for the i^{th} individual (CL_i) was estimated using equation 1.

$$CL_i = \theta_{pop} * \exp(\eta_i) \quad \text{(Eq. 1)}$$

where θ_{pop} represents the population value for MPA clearance, and η represents the between-patient random effect with mean 0 and variance ω^2. Within-patient variability of the pharmacokinetic parameters was also estimated. When estimating both within- and between-patient variability for clearance, equation 1 becomes equation 2:

$$CL_{ij} = \theta_{pop} * \exp(\eta_i + \kappa_j) \quad \text{(Eq. 2)}$$

where CL_{ij} is the clearance of MPA for i^{th} individual on the j^{th} occasion, and κ is the within-patient random effect with mean 0 and variance π^2.[14] The difference between the k^{th} observed MPA concentration of the i^{th} individual (C_{obsik}) and its corresponding model-predicted MPA concentration (C_{predik}), was estimated with an additional error model (equation 3):

$$\ln C_{obsik} = \ln C_{predik} + \varepsilon_{ik} \quad \text{(Eq. 3)}$$

where ε is the residual random error with mean 0 and variance σ^2.

The adequacy of the developed NONMEM models was evaluated using the precision of the parameter estimates and goodness-of-fit plots. Standard errors for the estimated population parameters (both for pharmacokinetic parameters and for random effects) were generated in NONMEM via the covariance option. Goodness-of-fit can be demonstrated in plots of observed concentrations versus model-predicted or Bayesian-predicted concentrations or plots of weighed residuals (WRES) versus time [15]. Bayesian predicted concentrations were obtained with the posthoc option in NONMEM. Goodness-of-fit plots were generated in Xpose version 3.010, an S-PLUS based (Version 6.1, professional edition, first release; Insightful Corp, Seattle, WA, USA) modelling aid [16].

Covariate Analysis

To explain pharmacokinetic variability between and within-patients, relationships were investigated between pharmacokinetic parameters and patient characteristics. Covariates tested were patient age, gender, race, weight, diabetic status [17], creatinine clearance (CrCl), plasma albumin concentration, liver enzymes AST and ALT, bilirubin, haemoglobin, mycophenolate mofetil dose and daily cyclosporin dose. Creatinine clearance was calculated using the Cockroft and Gault formula [18]. For categorical variables all data were available. For continuous variables on average 9% (range: 0–16.8%) of data were missing. For the covariates plasma albumin concentration, AST, ALT and bilirubin, more than 10% of data were missing. A missing value for an individual was replaced by the nearest available value in time for that individual. If there was not an available value within 1 month, the missing value was replaced by the corresponding median of the total population.

Individual Bayesian estimates of the pharmacokinetic parameters were generated and relationships between individual parameters and the covariates were visually inspected and investigated in NONMEM. A two-stage approach was used:

1. In the first step, all different covariates were introduced in the structural model separately and tested for their significance. Covariates were introduced in a multiplicative way. Categorical variables, such as gender, were modelled as shown in equation 4:

$$CL_{ij} = \theta_{pop} * \theta^{gender} \quad \text{(Eq. 4)}$$

where θ^{gender} is the fractional change in clearance in males (in females gender = 0 and in males gender = 1). Continuous variables, such as weight (WT), were modelled around the median value in the population (equation 5):

$$CL_{ij} = \theta_{pop} * (WT/68)^{\theta_{WT}} \quad \text{(Eq. 5)}$$

where median weight of the total population is 68 kg and θ_{WT} is an exponential.

Whether inclusion of a covariate significantly improved the fit was determined by two criteria. Firstly, the likelihood ratio test. For two hierarchical models, the difference between the minimum value of objective function (OFV), produced by NONMEM for both models, is approximately χ^2 distributed with degrees of freedom equal to the difference in the number of estimated parameters in the two models. The model with the lower OFV has a better goodness-of-fit. A p-value of 0.001 was considered to be statistically significant, which corresponds with a decrease in OFV of 10.8 units with one degree of freedom [19]. Secondly, a reduction in unexplained between- and within-patient variability was evaluated as a criterion for covariate inclusion.

2. In the second step, all covariates selected during the first step were included in an intermediate model. Covariates were excluded separately from the intermediate model (backward elimination). If the elimination of a covariate caused an increase of the OFV of at least 10.8 units ($p<0.001$, 1 degree of freedom), the covariate remained in the model. The result of the backward elimination procedure is the final model.

Model Validation

The stability and the performance of the final model were checked with an internal validation of the final model, using the bootstrap resample technique [20]. During a bootstrap procedure, approximately 65% of the original data are resampled with replacement, which produces different combinations of datasets. The final model is fitted to each artificial sample to obtain estimates for all parameters (fixed and random effects). The validation of the final model was performed using the bootstrap option in the software package Wings for NONMEM (Dr N. Holford, version 405, november 2003, Auckland, New Zealand).

Statistical Analysis

All statistical analysis, other than the evaluation of the model, were performed with the software package SPSS 10.1 for Windows (SPSS Inc. Chicago, IL, USA). For the comparisons between groups, the Mann-Whitney U test was used. For paired data analysis, the Wilcoxon signed rank test was applied. A p-value <0.01 was considered to be significant given the large sample size.

RESULTS

Structural Model

A total of 6523 plasma concentration-time data obtained from 140 renal transplant recipients were analysed. Figure 1 shows characteristic pharmacokinetic profiles of MPA after oral mycophenolate mofetil administration, with a rapid increase in MPA concentration during the absorption phase, often preceded by a lag time, followed by a distribution and an elimination phase. The data were fitted to several structural models. The data fitted a two compartment model adequately. In a plot of WRES versus time, the residuals were randomly distributed around the axis WRES=0 and no trends were observed. Adding an extra peripheral compartment yielded a significantly lower OFV, but estimates for peripheral volumes of distribution became unrealistic with values of >10 000 L. Consequently, the three compartment model was rejected. The absorption phase was best described by a first-order absorption rate constant as this gave significantly better fit compared with a zero-order absorption rate constant. Although the likelihood ratio test cannot be applied in this situation, the OFV was 115.9 units lower with first-order absorption. Introduction of an absorption lag time in the model further improved the fit significantly; the OFV decreased with 410.0 units.

Between-patient variability could be estimated for the first-order absorption rate constant (k_a), central volume of distribution (V1), peripheral volume of distribution (V2) and clearance; corresponding values were 136%, 126%, 114% and 35%, respectively. The OFV of this model was 1613.9 units. Introduction of between-patient variability on lag time did not improve the model and produced imprecise values for this random effect, therefore it was not included in the model.

Introduction of within-patient variability for CL, k_a and V1 greatly improved the fit of the model. Corresponding values were 30%, 117% and 69%, respectively, and the OFV decreased from 1613.9 to 378.1 units. The estimated population pharmacokinetic parameters of the structural model are summarised in table 2 and goodness-of-fit plots are given in figure 2. The goodness-of-fit plots in figure 2a and 2b show a symmetrical and random pattern around the line of identity. However, plots of observed concentrations versus model predicted concentrations on the sampling time points 0.67 h and 1,25 h per AUC target group (not shown) provide evidence of a small underestimation of C_{max}.

Because this minor amount of model misspecification is within acceptable limits (99.4% of WRES fall between –3 and 3 and no structural deviations from the line WRES = 0 occur, figure 2c), the model is believed to describe the data adequately.

Table 2 Estimates for the structural and final model with their coefficients of variation (CV)

	Structural model OFV = 378.1 units		**Final model** OFV = -338.8 units		**1000 bootstrap replicates**	
Parameter	**Estimate (CV%)**		**Estimate (CV%)**		**Mean estimate (CV%)**	
PK parameters						
Ka (h-1)	3.8	(6.2)	4.1	(6.8)	4.1	(7.9)
V1 (L)	98	(9.2)	91	(7.2)	92	(7.2)
CL (L/h)	31	(5.1)	33	(5.4)	33	(5.5)
V2 (L)	302	(8.4)	237	(10)	239	(11)
Q (L/h)	44	(7.9)	35	(5.3)	35	(6.7)
T_{lag} (h)	0.21	(1.5)	0.21	(1.3)	0.21	(8.7)
Between-patient variability						
ηKa (%)	136	(14)	111	(15)	115	(18)
ηV1 (%)	126	(15)	91	(13)	91	(8.9)
ηCL (%)	35	(15)	31	(15)	31	(7.8)
ηV2 (%)	114	(26)	102	(25)	103	(13)
Within-patient variability						
κKa (%)	117	(13)	116	(11)	117	(5.7)
κV1 (%)	69	(14)	53	(17)	53	(11)
κCL (%)	30	(8.8)	20	(11)	20	(5.5)
Residual variability						
Additive error (σ)	0.45	(2.4)	0.45	(2.3)	0.44	(2.4)
Covariates						
V1 θ CrCl	-	-	-0.62	(16)	-0.62	(16)
V1 θ Albm	-	-	-1.13	(23)	-1.15	(23)
CL θ Gender	-	-	1.11	(4.3)	1.10	(4.4)
CL θ CrCl	-	-	-0.12	(30)	-0.12	(31)
CL θ Albm	-	-	-1.07	(11)	-1.06	(11)
CL θ CsA dose	-	-	0.31	(11)	0.32	(11)

Ka = first order absorption constant, V1 = central distribution volume, CL = clearance, V2 = peripheral distribution volume, Q = intercompartment clearance, T_{lag} = lag time, η = between-patient variability, κ = within patient variability, θ = estimate of covariate, CrCl = creatinine clearance, Albm = plasma albumin concentration, CsA = cyclosporine.

Figure 1 Characteristic concentration-time profiles of mycophenolic acid (MPA) for the: (a) low area under the plasma concentration-time curve (AUC) [16.1 mg*h/L] target group; (b) intermediate AUC (32.2 mg*h/L) target group; and (c) high AUC (60.6 mg*h/L) target group.

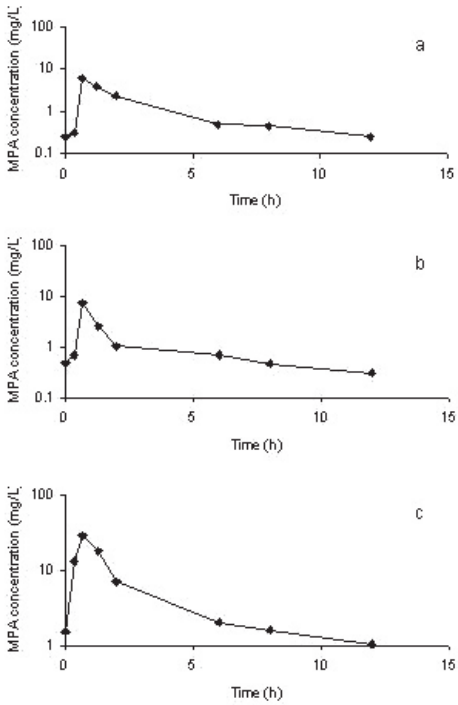
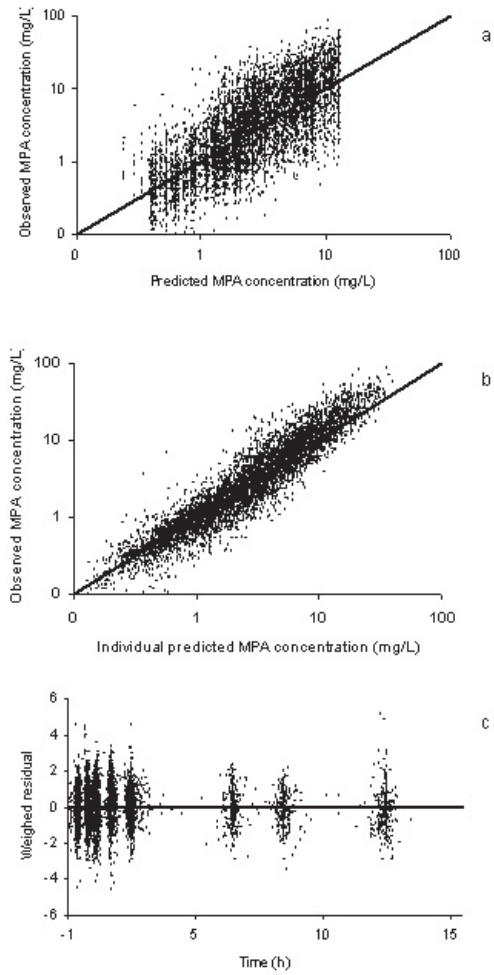


Figure 2 Goodness-of-fit plots for the structural model: (a) model predicted mycophenolic acid (MPA) concentration vs observed MPA concentration; (b) individual Bayesian-predicted MPA concentration vs observed MPA concentration; and (c) sample time vs weighed residuals. The solid lines in a and b represent the line of identity and in c the line y = 0.



Covariate Analysis

The analysis of the relationship between patient factors and pharmacokinetic parameters produced an intermediate model with the following correlations: CrCl and mycophenolate mofetil dose for k_a; diabetes mellitus, CrCl, plasma albumin concentration and mycophenolate mofetil dose for V1; diabetes mellitus, gender, CrCl, plasma albumin concentration, dialy ciclosporin dose, weight and ALT for CL; and plasma albumin concentration for V2. During the backward elimination procedure it appeared that CrCl and mycophenolate mofetil dose for k_a, diabetes for V1, diabetes and weight for CL and plasma albumin concentration for V2 did not result in a significant increase of OFV when excluded from the intermediate model. These relationships were therefore not incorporated in the final model. All other variables caused a significant rise of OFV when eliminated from the model. Plots of mycophenolate mofetil dose versus V1 and ALT versus CL both showed positive correlations. Both were excluded from the model because no pharmacologically sound explanation could be given for the relationship between ALT and CL, and because the relationship between mycophenolate mofetil dose and V1 was very weak, albeit statistically significant, and without clinical significance. Population V1 and CL (table 2) were described by the following equations in the final model (equation 6 and 7):

$$V1 = 91 * (CrCl/48)^{-0.62} * (Albm/30)^{-1.13} \quad \text{(Eq. 6)}$$

$$CL = 32.5 * (CrCl/48)^{-0.12} * (Albm/30)^{-1.07} * (CsA/450)^{0.31} * 1.11^{gender} \quad \text{(Eq. 7)}$$

From these equations it appears that V1 typically increases from 80 to 242 L when CrCl falls from 60 to 10 mL/min, assuming the plasma albumin concentration is 30 g/L. When the plasma albumin concentration increases from 30 to 50 g/L, V1 decreases from 92 to 53 L. Based on equation 7, it appears that males have an 11% higher MPA clearance than females. Furthermore, a reduction in CrCl from 60 to 10 mL/min, with a daily ciclosporin (CsA) dose of 450 mg and plasma albumin concentration of 30 g/L, causes an increase in MPA clearance from 32 L/h to 39 L/h (figure 3a). When the plasma albumin concentration rises from 30 to 50 g/L, MPA clearance decreases from 33 to 18 L/h (figure 3b). A ciclosporin dose change from 1000 mg/day to 700 mg/day produces a decrease of MPA clearance from 41 to 33 L/h (figure 3c).

The inclusion of these covariates in the final model explained both between- and within-patient variability for V1 and CL when compared with the structural model (table 2). The estimate for between-patient variability for V1 decreased from 126% to 91% and for CL from 35% to 31%. The estimated within-patient variability was reduced from 69% to 53% for V1 and from 30% to 20% for CL.

All parameters were estimated with an acceptable coefficient of variation of ≤30%. The goodness-of-fit plots of the final model are presented in figure 4.

Model validation

The results of 1000 bootstrap replicates are summarised in table 2. The mean estimates resulting from the bootstrap procedure are very similar to the population estimates of the final model. This means that the estimates for the fixed and random effects in the final model are accurate and that the model is stable.

Figure 3 Correlations for mycophenolic acid (MPA) clearance with (a) renal function (creatinine clearance [CrCl]); (b) plasma albumin concentration; and (c) daily cyclosporin dose, as identified in the final model. The solid lines represent the correlation estimated by the population pharmacokinetic model.

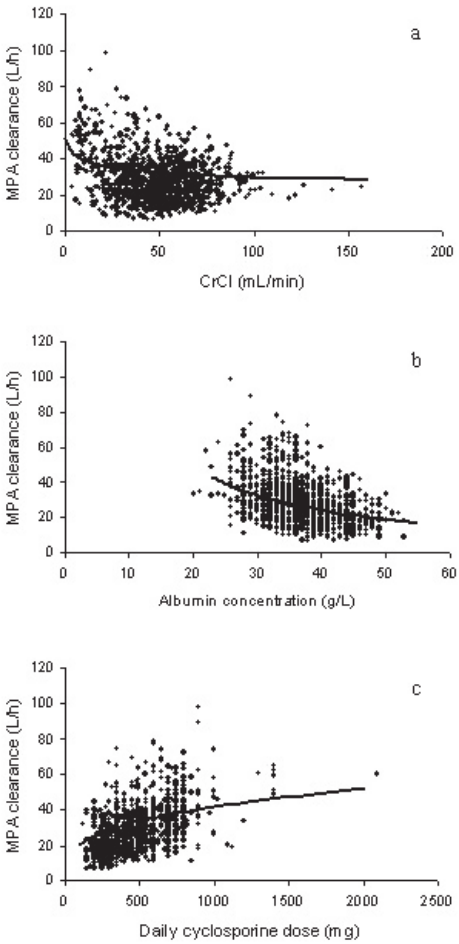
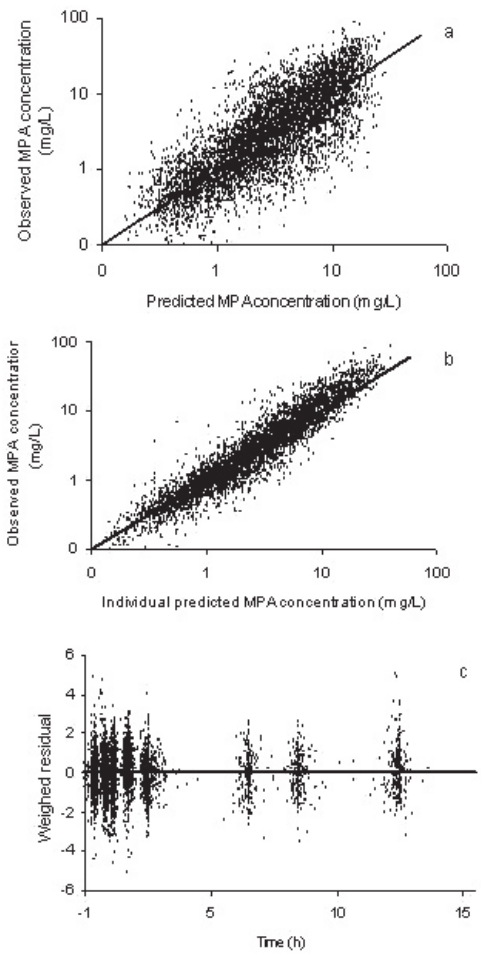


Figure 4 Goodness-of-fit plots for the final model: (a) predicted mycophenolic acid (MPA) concentration vs observed MPA concentration; (b) individual Bayesian-predicted MPA concentration vs observed MPA concentration; and (c) sample time vs weighed residuals. The solid lines in a and b represent the line of identity and in c the line y = 0.

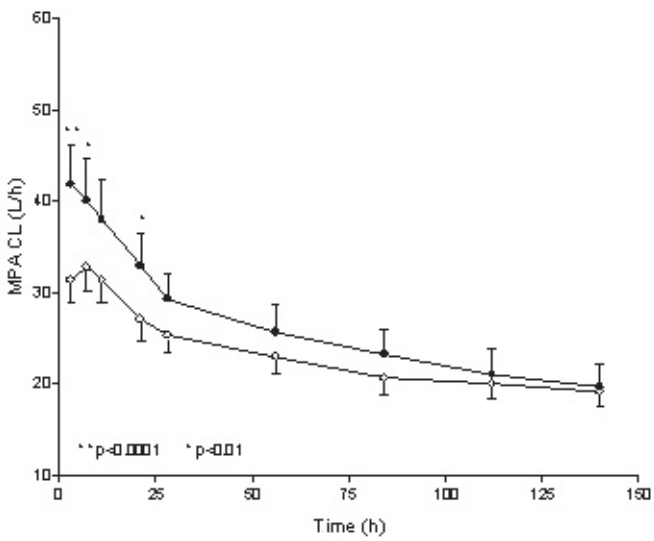


Analysis of the Influence of Renal Function on the Decrease of Mycophenolic acid Clearance

The covariate analysis revealed that renal function significantly correlates with MPA clearance. To investigate whether this effect plays a role in the described decrease in clearance for MPA during the first weeks after transplantation [8], the study population was divided into two groups: those with CrCl <25 mL/min lower (n=56) and those with CrCl >25 mL/min (n=84) on day 3 after transplantation. The cut-off point of CrCl 25 mL/min was based on visual inspection of the plotted relationship between CrCl and MPA clearance in figure 3a. The group with CrCl <25 mL/min contained 21 of the 23 patients who experienced delayed graft function. The course of the Bayesian estimate of clearance over time was studied for both groups.

The results are shown in figure 5. Renal transplant patients with CrCl <25 mL/min showed a significantly higher mean MPA clearance on days 3 (34% higher; $p < 0.0001$), 7 (22% higher; $p = 0.005$), and 21 (22% higher; $p = 0.006$) after transplantation compared with patients with CrCl >25 mL/min. Also on days 11 and 28 after transplantation, MPA clearance was higher in patients with CrCl <25 mL/min (21% higher on day 11; $p = 0.014$, and 15% higher on day 28, $p = 0.026$), although not with a significance level of $p < 0.01$. Both groups experienced a significant decrease in MPA clearance during the first 28 days after transplantation ($p < 0.0001$ for both groups). Patients with a CrCl <25 mL/min on day 3 had a significant larger fall in clearance during the first 28 days compared with patients with a CrCl >25 mL/min (10 ± 9.6 L/h respectively 5.2 ± 8.1 L/h, $p = 0.003$).

Figure 5 Mean time-course of mycophenolic acid (MPA) clearance for patients with creatinine clearance (CrCl) < 25 mL/min (n=56, closed circles) and CrCl > 25 mL/min (n=84, open circles) on day 3 after transplantation. Error bars represent 95% confidence interval limits.



DISCUSSION

The pharmacokinetics of MPA following oral administration of the prodrug mycophenolate mofetil exhibit considerable between- and within-patient variability. This study focused on developing a clinically applicable population pharmacokinetic model for MPA to quantify between- and within-patient variability and analyse relationships between pharmacokinetic parameters and patient demographics and biochemical factors with the aim of explaining the observed variability.

The pharmacokinetics of MPA in 140 patients was described with a two compartment model with a time-lagged first-order absorption rate. Plots of observed concentrations versus model-predicted or Bayesian-predicted concentrations demonstrated an adequate goodness-of-fit. Fixed and random parameters were estimated with acceptable precision and residual variability was small (figures 2 and 4). Nevertheless, model diagnostics showed a slight underestimation of maximum MPA concentrations (C_{max}). The likely reason for this underestimation is that with orally administrated drugs, conventional compartmental models with a lag time and a first- or zero-order absorption rate constant are often not able to accurately predict a rapid initial increase in plasma concentration [21]. More complex and mechanistic models may be necessary to accurately describe the absorption phase [21]. Since the underestimation of C_{max} was small and within acceptable limits, the model was believed to be sufficiently accurate to describe MPA pharmacokinetics and its variability. The validity of the derived model was confirmed by the results of the bootstrap procedure yielding similar values for all parameters and their corresponding precision.

The pharmacokinetic model did not include enterohepatic recirculation (EHC) of MPA, although it has been established that up to 60% of a mycophenolate mofetil dose undergoes recirculation in healthy individuals [4]. EHC is responsible for the secondary rise in MPA concentration typically occurring approximately 6-12 hours after oral administration of mycophenolate mofetil [4]. However, in the plot of WRES versus time, no trends were observed in this time period and WRES values were randomly distributed around the WRES = 0 axis (figures 2c and 4c). As a result, it appears that EHC does not influence the pharmacokinetics of MPA to a large extent in the renal transplant population studied.

The developed population pharmacokinetic model demonstrated considerable between-patient variability among the pharmacokinetic parameters. Only a moderate amount of between-patient variability of CL and V1 could be explained by inclusion of the covariates renal function, plasma albumin concentration and daily ciclosporin dose: 11% en 28% respectively. Unexplained between-patient variability of CL was 31%. Between-patient variability has also been analysed in other studies. One study reported the MPA AUC to range from 7.5 to 94.7 mg*h/L during the first weeks after renal transplantation [5]. In that same period, a paediatric study found that between-patient MPA AUC could vary 28% and 37%, while in another study of adult renal transplant recipients, 32-58% between-patient variability was observed [22,23].

In addition to between-patient variability in exposure, considerable within patient-variability in exposure during the first weeks after transplantation has also been reported in other studies [8,23,24]. In the present analysis, 33% of the within-patient variability in clearance was explained by the included covariates. The unexplained within-patient variability for CL was small, with a value of 20% in the final model.

When estimates of between- and within-patient variability of pharmacokinetic parameters are available, one might speculate on the usefulness of TDM of mycophenolate mofetil therapy. With support of TDM, between-patient variability in exposure to the drug is greatly reduced and patients can be targeted to a particular therapeutic window. Howver, large within-patient variability reduces the efficacy of TDM since these variations in time cannot be controlled. The present study demonstrated

a large between-patient variability and relatively small within patient variability of clearance, taking the identified covariates into account. This suggests that TDM may substantially reduce the observed differences in MPA exposure between individuals and may be useful in obtaining AUC values in the range of 30-60 mg*h/L, which is currently proposed [11].

The covariate analysis identified relationships between V1 and renal function and plasma albumin concentration, and between clearance and renal function, plasma albumin concentration, daily ciclosporin dose and gender. The observation that clearance increases with impaired renal function may be explained by reduced protein binding of MPA caused by uraemia, as well as MPAG accumulation [5]. Both processes may produce an increased free fraction of MPA, which in turn may lead to increased metabolism to MPAG since MPA is believed to have a low to intermediate extraction ratio [4]. The dependence of MPA CL on protein binding has been suggested by several studies [24,25], and is confirmed in the present analysis. However, the correlation between renal function and clearance only appears to be of clinical significance when CrCl is very low (<25 mL/min [figure 3a]). A decrease in CrCl from 90 to 50 mL/min induces only a modest increase in MPA clearance from 30 L/h to 33 L/h, whereas a fall from 50 to 10 mL/min increases MPA clearance from 33 L/h to 39 L/h. The controversy about the influence of renal function on MPA clearance in the literature, where some studies have found a relationship while others have not [12,24-27], may be due to the observation that MPA clearance is only influenced with severely impaired renal function. The majority of patients with CrCl <25 mL/min suffered from delayed graft function in the first week(s) after transplantation. At this time, patients then may have low plasma albumin concentrations, metabolic acidosis and uraemia, resulting in an increase in MPA free fraction and thus in higher MPA clearance. Subsequently, as the condition of patients improve, normal nutritional status and normal albumin levels are attained. The result may be that free MPA concentrations are not influenced, to a large extent, even when renal function is moderately impaired.

The negative correlation between plasma albumin concentrations and MPA clearance is in accordance with the reported intermediate extraction ratio for MPA, which suggests that its pharmacokinetics depend on free fraction [4,28]. This observed relationship is confirmed by results from other studies. An *in vitro* study showed that increasing albumin concentrations lead to a decreased free MPA fraction [7]. This relationship has been confirmed in a paediatric renal transplant population [26]. Furthermore, a study using rat livers demonstrated that MPA clearance increased with decreasing albumin levels [29].

Inclusion of the relationships between clearance and both creatinine clearance and plasma albumin concentration improved the model. Although creatinine clearance and albumin concentrations are related (albumin levels increase with recovering renal function), the correlation was weak in this study (r^2=0.124) and exclusion of one of these relationships significantly worsened the model, which indicates that both factors independently influence MPA clearance. The dependence of MPA clearance on renal function and albumin levels is further strengthened by the observed relationships between V1 and renal function and plasma albumin concentrations. These relationships revealed that impaired renal function or decreased plasma albumin concentrations lead to a rise in V1, potentially as a consequence of transfer of the increased free MPA quantity into peripheral tissues. This produces relatively lower total plasma concentration and a higher estimate of V1. Unfortunately, from our study it is uncertain how renal function and plasma albumin concentrations affect free MPA concentrations, since free MPA levels were not measured.

Concomitant immunosuppressive medication, such as ciclosporin, has been reported to interact with MPA clearance. Ciclosporin is believed to decrease MPA AUC [30,31]. In the present study, an effect of CsA was confirmed by demonstrating a positive correlation between ciclosporin daily dose and MPA

clearance. A logical explanation for this correlation may be the inhibitory effect of ciclosporin on EHC of MPA [32]. Since all patients took ciclosporin as concomitant immunosuppressive therapy, this may, at least in part, explain the adequate goodness-of-fit of the model without including EHC. As EHC seems to be present more profoundly when mycophenolate mofetil is combined with tacrolimus [32], a similar population pharmacokinetic analysis is warranted in a tacrolimus-mycophenolate mofetil treated renal transplant population.

As a clinical consequence of the covariate analysis, mycophenolate mofetil dosing may be optimised as exposure to MPA may be predicted more accurately when plasma albumin concentrations, ciclosporin daily dose, and renal function are taken into account. Besides, a change in these factors provides an indication to efficiently apply TDM of MPA. Both mycophenolate mofetil dose optimisation and efficient application of TDM may contribute to a lower risk for acute graft rejection. It is important to note that free MPA concentrations, which were not measured in this study, may be constant with hypoalbuminemia or severely impaired renal function, through an increased free MPA fraction and an increased MPA clearance. Since free MPA is believed to be the pharmacologically active moiety, mycophenolate mofetil dose increases based on measurement of total MPA would not be warranted in such cases. The influence of the identified covariates on free MPA fraction and free MPA concentrations needs to be further investigated in order to be able to make sound recommendations about TDM of MPA and mycophenolate mofetil dose adjustments in patients with severe renal impairment or decreased plasma albumin concentrations.

Recently, Shum et al. [33] have performed a population pharmacokinetic analysis of MPA on the basis of data from 22 patients during the first month after renal transplantation. With a similar structural pharmacokinetic model as in the present study, between-patient variability for both k_a and V1 was estimated to be lower, whereas within-patient variability for k_a and V1 was estimated to be higher. For clearance, both estimates for variability were lower with corresponding values of 20% and 13% and no relationships were assessed between patient factors and clearance. The observed differences with the present study may be explained by the lower number of patients and the shorter follow-up time in the study by Shum et al. [33] Another population pharmacokinetic analysis of MPA by Le Guellec et al. [34] was performed in stable renal transplant recipients at least 6 months after surgery. MPA clearance was estimated to be 15.7 L/h for a typical patient weighing 64 kg with a between-patient variability of 28%. The value for MPA clearances matches well with the result found in this study for the median MPA clearance at day 140 after transplantation, i.e. 18.1 L/h.

With regard to the change of MPA clearance over time, the findings of Shaw et al. [24] contrast with the suggestion from several studies [6,10] that MPA clearance decreases to the same extent in all renal transplant patients. Shaw et al. showed that patients who do not have a well functioning graft immediately after transplantation experience the most pronounced decrease in MPA clearance [24]. The negative correlation between MPA clearance and renal function observed in this study confirms that patients with impaired renal function shortly after transplantation will experience a significant change in clearance when renal function improves in the first post-operative weeks. Interestingly, Shaw et al. [24] did not find a significant change in MPA clearance in patients with immediate graft function following transplantation, whereas our results show a significant change in the group with CrCl >25 mL/min, albeit less pronounced than in the patient with CrCl <25 mL/min (figure 5). Other studies [22,27] also found a decrease in MPA clearance regardless of renal function during the first period after renal transplantation. This change may be due to changes in the plasma albumin concentration, as well as tapering of ciclosporin daily dose in the first period after transplantation.

CONCLUSION

Through the development of a population pharmacokinetic model, more insight was obtained in the pharmacokinetics of MPA after oral intake of mycophenolate mofetil. Between- and within-patient variability were quantified and in part explained by correlations between MPA clearance and renal function, plasma albumin concentration and ciclosporin daily dose and between V1 and renal function and plasma albumin concentration. The identified correlations offer a possible explanation for the decreasing MPA clearance observed in renal transplant recipients in the first weeks after transplantation. The clinical relevance of changing MPA clearance and V1 in a situation of altered renal function or plasma albumin concentration remains to be investigated. Nevertheless, the identified relationships may help to apply TDM of MPA more efficiently and to predict MPA concentrations resulting from a certain mycophenolate mofetil dose more accurately, thereby reducing the variability in MPA concentration within and between individuals.

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Chapter 2.2

Explaining Variability in Mycophenolic Acid (MPA) Exposure to Optimise Mycophenolate Mofetil Dosing: A Population Pharmacokinetic Meta-Analysis of MPA in Renal Transplant Recipients

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ABSTRACT

Large between- and within-patient variability has been observed in the pharmacokinetics of mycophenolic acid (MPA). However, conflicting results exist about the influence of patient characteristics that explain the variability in MPA exposure. This population pharmacokinetic meta-analysis of MPA in renal transplant recipients was performed to explore whether race, renal function, albumin level, delayed graft function (DGF), diabetes mellitus and co-medication are determinants of total MPA exposure. 13,346 MPA concentration-time data points from 468 renal transplant patients who participated in six clinical studies were combined and analysed retrospectively. Sampling occasions ranged from day 1 after transplantation to 10 years after transplantation. Concentration-time data were analysed with nonlinear mixed effect modeling (NONMEM). Exposure to total MPA, as determined by MPA clearance, significantly increased with increasing renal function, albumin level and haemoglobin as well as decreasing cyclosporine pre-dose levels ($p<0.001$). These variables could explain 18% of the between- and 38% of the within-patient variability in MPA exposure. Differences in MPA exposure between patients with or without DGF or between patients of different races, are likely to be caused by the effect of renal function on MPA exposure. Diabetes mellitus did not have an effect on MPA exposure.

The clinical implication is that a change in renal function or albumin level provides an indication for therapeutic drug monitoring as MPA exposure may be altered. Patients in whom cyclosporine and MMF are combined may need higher MMF doses especially during the early phase post-transplant than currently recommended for optimal MPA exposure.

INTRODUCTION

Mycophenolate mofetil (MMF) is an immunosuppressive drug successfully used in solid organ transplantation to prevent acute allograft rejection [1,2]. MMF is a prodrug of mycophenolic acid (MPA), which exhibits rapid and almost complete absorption from the gut. MPA has extensive plasma albumin binding (98%) and is metabolised by uridine glucuronosyl transferase enzymes into the pharmacologically inactive glucuronide metabolite (MPAG) [3-5]. The pharmacokinetics of MPA are further characterised by an enterohepatic recirculation, in which MPAG is excreted into bile and deglucuronidated in the gut back to MPA [3].

Low rates of acute rejection and long term patient survival have been achieved with MMF when used in a standard dose recommendation of 1 gram twice daily for adults. A number of pharmacokinetic studies have shown an increased risk for acute rejection in patients with lower MPA exposure, suggesting that efficacy may improve by adjusting the dose based on plasma concentrations. Based on these studies a target window has been adopted for MPA exposure (area-under-the-curve (AUC) values between 30 and 60 mg*h/L) [6-8]. Accumulating evidence suggests that this target is not reached in every patient with the standard MMF dose, with some studies reporting a 10-fold between-patient variability of MPA exposure, changes of exposure over time with a fixed MMF dose and influence of co-medication [4,9-11]. Consequently, individualisation of MMF dose may be necessary to achieve adequate MPA exposure in every patient.

By explaining between-patient variability in MPA pharmacokinetics and identifying the patient characteristics that significantly influence MPA exposure, rational decisions on optimal dosing can be achieved [12]. Earlier pharmacokinetic studies have already attempted to correlate MPA exposure to several explanatory factors. For example, some studies found that impaired renal function and low albumin levels result in high total MPA clearance and thus low total MPA exposure [5,9,11,13,14],

although this could not be confirmed in all studies [15-20]. Also with regard to the effect of the use of co-medication [3,10,18,21,22], diabetes mellitus [23,24], body weight [11,13,17,20] age [5,11,18-20], gender [11,20,23] and race [9,23,25] contrasting results have been obtained. Most of these studies were underpowered, based on MPA pre-dose levels only, and some studies did not adequately control for confounding factors. Consequently, for most variables it is not clear if and to what extent they influence MPA exposure and whether individualisation of the MMF dose should depend on these variables.

Population pharmacokinetic meta-analysis are known to be very powerful and can reliably estimate the determinants of pharmacokinetic variability, thereby explaining between-patient differences in drug exposure [26,27]. An important advantage of a population pharmacokinetic approach is that it allows pharmacokinetic data sets originating from several studies with different sampling time points to be combined. In this study, a population pharmacokinetic meta-analysis of MPA in renal transplant recipients was performed to explore whether race, age, gender, weight, renal function, albumin level, delayed graft function (DGF), diabetes mellitus and the use of antimicrobial agents, gastric pH modulators, cyclosporine and corticosteroids can explain variability in MPA exposure between (subgroups of) patients.

PATIENTS AND METHODS

Studies

Total MPA concentration-time data from 468 renal transplant patients who participated in six different studies were combined and analysed retrospectively. All data were provided by Roche Laboratories Inc. Details of these studies have been previously published elsewhere [6,7,23,28-30]. Per study, the number of patients from whom samples were drawn for pharmacokinetic analysis, the MMF starting doses, the occasions of pharmacokinetic assessment after transplantation, the time of sampling after MMF administration and the concomitant used immunosuppressive agents are summarised in table 1.

Data and definitions

Data on total MPA concentrations, timing of MPA sample drawing and MMF dosing history from the six studies were pooled in one dataset. Data were also collected on patient characteristics, routine laboratory measurements, co-medication, co-morbidity like diabetes mellitus and DGF for every sampling occasion in all patients. Pre-transplant diabetes mellitus was defined as the use of antidiabetic drugs within 60 days prior to transplantation, or a medical history of diabetes mellitus. DGF was defined as the need for dialysis in the first two weeks after transplantation. Three categories for race were defined: Caucasian, Black and other. The use of co-administered drugs was scored as 1 when the drug was used on the day of pharmacokinetic assessment, otherwise co-medication was scored as 0. The use of antiviral agents consisted of acyclovir or ganciclovir. Patient characteristics and biochemical parameters are summarised in table 2.

Table 1 Description of studies used for the population pharmacokinetic meta-analysis with regard to pharmacokinetic properties.

Study (Reference)	Number of subjects	MMF starting dose (mg twice daily)	Time of PK assessment after transplantation	Time of sampling after oral MMF administration (hours)	Concomitant immunosuppression
Unpublished study	18	1000, 1500 or 1750	Days 1 and 20	Pre-dose, 0.5, 1, 2, 4, 8 and 12	Prednisone Cyclosporine§
Sollinger [28] US MMF Study Group [29]	62	1000 or 1500	Days 1 and 5, hospital discharge (range: day 6-21)	Pre-dose, 0.5, 1, 2, 4, 8 and 12	Prednisone Cyclosporine‡
Van Gelder et al. [6] Hale et al. [7]	141	450, 950 or 1700*	Days 3, 7, 11 and 21, Months 1, 2, 3, 4 and 5	Days 3, 7 and 11: Pre-dose, 0.33, 0.67, 1.25, 2, 6, 8 and 12 Thereafter: Pre-dose, 0.33, 0.67, 1.25 and 2	Prednisone Cyclosporine¶
Ekberg et al. [30]	44	1000	Days 4 and 7 Months 1, 3 and 6	Pre-dose, 0.33, 0.67, 1.25, 2, 3, 4, 6, 8 and 12. Day 4 only pre-dose	Prednisone Cyclosporine¶ (n=14) or sirolimus (n=30) Daclizumab
Submitted for publication	118	750, 1000	Day 7 Months 3, 7 and 12	Pre-dose, 0.33, 0.67, 1.25, 2, 3, 4, 6, 8 and 12	Prednisone Cyclosporine# Daclizumab#
Pescovitz et al. [23]	85	1000, 1250 or 1500	>6 months (range: month 6 - year 10)	Pre-dose, 0.33, 0.67, 1.25, 2, 3, 4, 6, 8, 12	Prednisone Cyclosporine¶

§ In the unpublished study, cyclosporine was initiated when creatinine levels dropped below 3 mg/dL. ‡ In the study by Sollinger [28] and by the US MMF Study Group [29], cyclosporine was initiated after the first week. # The study submitted for publication was a three arm study: one arm with standard doses of cyclosporine (pre-dose levels for the first 4 months of 150-300 ng/mL, thereafter 100-200 ng/mL), one arm with low dose cyclosporine (pre-dose levels of 50-100 ng/mL) and standard dose dacluzimab and one arm in which cyclosporine was given in a low dose for the first three months, then cyclosporine was withdrawn over a three month period and standard dose daclizumab. ¶ In the remaining studies [6,7,23,30], cyclosporine was used according to routine practice. *In the study by Van Gelder et al. [6] and by Hale et al. [7] mycophenolate mofetil dose was based on area-under-the-curve (AUC) measurements to obtain target exposure in three predefined groups (low AUC target group [target AUC: 16.1 mg*h/L]; intermediate AUC target group [target AUC: 32.2 mg*h/L]; high AUC target group [target level: 60.6 mg*h/L]). Mycophenolate mofetil was dispensed as tablets of 250 mg, but to reach target exposure as closely as possible, the dose could be fine-tuned with capsules of 50 mg mycophenolate mofetil.

PK = pharmacokinetic

Table 2 Patient demographics and biochemical parameters

Characteristics		**Day 0-4¶**	**Month 1**	**Month 6**	**Year 1**
Gender					
Male	n=	157	119	87	104
Female	n=	89	69	47	67
Race					
Caucasian	n=	217	168	121	131
Black	n=	17	7	4	37
Other	n=	12	13	9	3
Diabetes mellitus	n=	49	17	15	16
DGF	n=	34	-	-	-
Use of antacids	n=	56	24	0	1
Use of proton pump inhibitors	n=	7	13	0	0
Use of H_2-antagonists	n=	66	82	3	5
Use of antiviral agents	n=	68	8	1	0
Use of sirolimus	n=	21	27	17	2
Age (years)		50 (18-72)	50 (19-70)	49 (28-70)	52 (22-73)
Body weight (kg)		71 (37-151)	68 (38-151)	80 (42-151)	75 (49-122)
Serum creatinine (µmol/L)		424 (66-1379)	128 (53-913)	124 (62-195)	125 (52-221)
Creatinine clearance (mL/min)		19 (4-132)	55 (7-203)	71 (44-132)	64 (34-113)
Plasma albumin (g/L)		35 (23-51)	35 (26-50)	36 (29-45)	42 (31-48)
Serum ALT (U/L)		17 (2-653)	17 (4-144)	25 (10-128)	20 (11-1759)
Serum total bilirubin (mg/dL)		0.5 (0.2-3.0)	0.6 (0.1-1.9)	0.5 (0.1-1.6)	0.7 (0.2-3.3)
Serum alkaline phosphatase (U/L)		64 (17-870)	86 (25-221)	99 (46-218)	171 (41-347)
Red blood cell count (x10^{12}/L)		3.2 (1.5-4.8)	3.4 (2.1-4.9)	4.3 (3.5-5.9)	4.4 (3.7-9.5)
Haemoglobin (g/dL)		9.7 (4.9-17)	11 (6.7-15)	12 (9.6-18)	13 (7.8-18)
Prednisone daily dose (mg)		30 (20-1365)	19 (7.5-35)	10 (0-10)	9.4 (0-10)
Cyclosporine dose					
Daily dose in mg		530 (0-1000)	350 (0-1400)	50 (0-200)	138 (0-300)
Daily dose in mg/kg		6.0 (0-18)	6.4 (0-22)	0.5 (0-3.6)	1.8 (0-6.6)
Cyclosporine pre-dose level (ng/mL)		171 (0-806)	237 (0-571)	93 (0-316)	155 (0-1337)
Patients not using cyclosporine	n=	102	27	17	34
MMF dose					
MMF dose in mg twice daily		1150 (400-2200)	1000 (250-2200)	1000 (1000-1000)	1000 (250-1250)
MMF dose in mg/kg twice daily		15 (4.8-36)	15 (3.9-45)	11 (2.2-18)	14 (3.8-21)

Data are presented as median (range) for four sampling occasions after renal transplantation: day 0-4, month 1, month 6 and year 1. In total, data were collected from 468 renal transplant recipients participating in 6 clinical studies.

¶ For demographic description of the study population during day 0-4 one value per variable per patient was used, namely the one measured on the day of pharmacokinetic assessment.

Because of different moments of pharmacokinetic assessment after transplantation in the studies, the number of individuals from whom data were available differs for the four presented occasions.

n = number of renal transplant recipients, MMF = mycophenolate mofetil, DGF = delayed graft function, ALT = alanine transferase, Normal values for creatinine: 65-115 µmol/L for males and 55-90 µmol/L for females, for plasma albumin: 35-50 g/L, for serum ALT: <41 U/L for males and <31 U/L for females, for serum total bilirubin: <1 mg/dL, for serum alkaline phosphatase: <120 U/L, for red blood cell count: 4.4-5.6x10^{12}/L for males and 3.9-4.9x10^{12}/L for females, for haemoglobin: 13.8-16.9 g/dL for males and 12.1-15.3 mg/dL for females.

Pharmacokinetic analysis

All data were analysed simultaneously using the nonlinear mixed effects modeling software program (NONMEM) (Version V, level 1.1, GloboMax LLC, Hanover, USA). NONMEM is a parametric nonlinear multiple measurements regression program designed for population pharmacokinetic analyses. This kind of analysis quantifies two types of population pharmacokinetic parameters based on linking dosage, time and observable patient features to drug concentrations [26,27,31,32]. The first type are fixed effect parameters, which quantify mean population pharmacokinetic parameters, or typical relationships between patient features like gender or race, and individual pharmacokinetic parameters. The second type are random effect parameters, which measure between-, and within-patient variability of pharmacokinetic parameters [32,33]. Using the first order method in NONMEM, the population pharmacokinetic parameters are calculated by simultaneously fitting all data to a pharmacokinetic model [32]. This means that NONMEM appropriately pools data across individuals, which makes the population parameter estimates less dependent on the number of samples per individual, while at the same time it allows easy combination of concentration-time data collected in different studies with different sampling schemes and on different moments after transplantation [27].

A more detailed description of the technical aspects of the methods used for pharmacokinetic modeling will be reported elsewhere [34]. Briefly, during the first step of the analysis a compartmental population pharmacokinetic model was developed describing the pharmacokinetics of MPA and quantifying between- and within-patient variability in MPA pharmacokinetics. Data were logarithmically transformed and residual variability was modeled additively [27]. Individual estimates of the pharmacokinetic parameters were obtained by Bayesian analysis.

The second step was the investigation of relationships between patient factors and individual estimates of the pharmacokinetic parameters. Patient factors tested were patient race, age, gender, weight, albumin level, alanine transferase (ALT), bilirubin, alkaline phosphatase, haemoglobin, red blood cell count, DGF, diabetes mellitus, cyclosporine dose, cyclosporine pre-dose concentration, MMF dose, corticosteroid dose and the use of antiviral agents, proton pump inhibitors, antacids, H_2-antagonists, sirolimus, and renal function. With regard to the latter, two estimates for renal function were tested: estimation of creatinine clearance according to the Cockcroft and Gault formula (C&G) [35] and estimation of the glomerular filtration rate with the abbreviated Modification of Diet and Renal Disease (aMDRD) method [36]. First, all different variables were tested in the model developed during the first step, in a univariate way. Whether a variable had a significant effect was determined with the likelihood ratio test. A p-value <0.001 was considered to be statistically significant. Second, a multivariate analysis (backward elimination procedure) was done to obtain the final model. The final model was refined by estimating between-patient variability in the relationships between patient factors and pharmacokinetic parameters. This variability parameter takes into account that a change in the value of a variable may not have the same effect on a pharmacokinetic parameter in all individuals [37].

MPA AUC values, normalised to 1000 mg MMF, were calculated based on the individual estimates for MPA clearance from the final model (equation 1):

MPA AUC (mg*h/L) = 1000 mg / MPA clearance (L/h) (Eq. 1)

Statistics

Statistical analyses were performed with the software package SPSS 11.5 for Windows (SPSS Inc. Chicago, IL, USA). For comparisons of continuous parameters between groups and within a group over time, repeated measures ANOVA was used. A p-value less than 0.05 was considered significant.

RESULTS

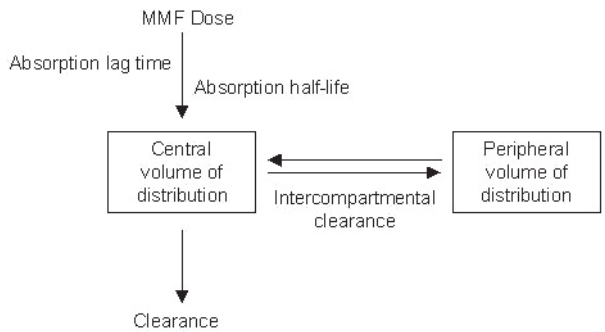
Data

In total, 13,346 MPA samples originating from 1894 concentration-time curves obtained from 468 renal transplant recipients were analysed. Sampling occasions varied from day 1 to day 3795 (>10 years) after renal transplantation and MMF doses ranged from 250 mg twice daily to 2200 mg twice daily. 884 MPA concentration-time curves originated from the first month after transplantation, while 280 pharmacokinetic profiles were taken after the first half-year.

Pharmacokinetic analysis

The model after the first step was a two compartment model with a lag-time preceding the absorption phase (figure 1).

Figure 1 Schematic presentation of the population pharmacokinetic model that best fitted the data, which was a two compartment model with time lagged first order absorption. MMF = mycophenolate mofetil.



The results of the uni- and multivariate analyses of relationships between pharmacokinetic parameters and patient factors are summarised in table 3. The correlations between pharmacokinetic parameters and C&G were statistically stronger as determined with the likelihood ratio test than correlations between pharmacokinetic parameters and aMDRD. For this reason C&G was used as measure for renal function and aMDRD was rejected.

After the multivariate analysis, significant relationships were found between C&G, albumin level, haemoglobin and cyclosporine pre-dose level and MPA clearance (table 3, all $p<0.001$). These correlations are reported as relationships with MPA AUC_{0-12} (normalised to 1000 mg MMF, equation 1), as clearance and dose are the only determinants of AUC_{0-12}. MPA AUC_{0-12} was higher when renal function was better: AUC_{0-12} was 36 mg*h/L with a C&G of 20 mL/min and 45 mg*h/L when C&G was 65 mL/min (figure 2a). A higher albumin level correlated with a higher MPA AUC_{0-12}: 42 mg*h/L when albumin level was 35 g/L and 48 mg*h/L with an albumin level of 42 g/L (figure 2b). Furthermore, AUC_{0-12} was higher with a haemoglobin of 12.5 mg/dL (AUC_{0-12} was 45 mg*h/L) compared to a haemoglobin of 10 mg/dL (AUC_{0-12} was 42 mg*h/L) (figure 2c). Finally, a lower cyclosporine pre-dose level correlated with a higher AUC_{0-12}: 45 mg*h/L with a cyclosporine pre-dose level of 150 ng/mL and 43 mg*h/L with a pre-dose level of 225 ng/mL (figure 2d). While the separate patient factors had a small to modest effect on MPA AUC_{0-12}, an almost doubling of AUC_{0-12} from 31 to 56 mg*h/L was found when the described effects of renal function, albumin levels, haemoglobin and cyclosporine pre-dose levels were combined.

Table 3 Relationships between pharmacokinetic parameters and patient factors

Pharmacokinetic parameter	Significantly correlated variables after univariate analysis*	Significantly correlated variables after multivariate analysis*
Absorption half-life:	C&G aMDRD Cyclosporine dose Use of H_2-antagonists	Cyclosporine dose
Central volume of distribution:	C&G aMDRD Albumin level Haemoglobin ALT Cyclosporine dose Use of antacids Use of antiviral agents	C&G Albumin level Use of antacids
Clearance:	C&G aMDRD Delayed graft function Albumin level Haemoglobin Red blood cell count ALT Alkaline phosphatase Caucasian race Cyclosporine dose Cyclosporine pre-dose level Corticosteroid dose Use of sirolimus Use of antacids Use of antiviral agents Use of H_2-antagonists	C&G Albumin level Haemoglobin Cyclosporine pre-dose level

Relationships between pharmacokinetic parameters and patient factors were tested using the model shown in figure 1. Relationships were first tested in a univariate way, whereafter the significantly correlating variables were included in a multivariate analysis (backward elimination procedure) to obtain the final model.
*All relationships between pharmacokinetic parameters and patient factors were significant at the level of $p<0.001$.
ALT = alanine transferase C&G = estimation of the creatinine clearance according to Cockcroft and Gault, aMDRD = estimation of the glomerular filtration rate according to the abbreviated Modification of Diet and Renal Disease method.

Table 4 Parameter estimates for the final model with their standard errors

Parameter	Population estimate, mean (SE)	Between-patient variability, %CV (SE)	Within-patient variability, %CV (SE)
Absorption half-life	0.17 h (0.012)	101 % (14)	116 % (12)
Central volume of distribution	69 L (4.0)	90 % (14)	71 % (8.3)
Clearance	23 L/h (0.54)	36 % (3.4)	21 % (2.1)
Peripheral volume of distribution	298 L (23)	-	-
Intercompartmental clearance	34 L/h (2.5)	60 % (13)	41 % (16)
Absorption lag time	0.24 h (0.0028)	-	-

%CV = coefficient of variation for variability, SE = standard error of parameter estimate. PK = pharmacokinetic.
Parameters were estimated taking into account the effect of cyclosporine dose on absorption half-life, the effect of renal function (creatinine clearance calculated according to Cockcroft and Gault), plasma albumin concentration and the use of antacids on central volume of distribution and the effect of renal function, albumin level, cyclosporine pre-dose level and haemoglobin on clearance. Estimates for between- and within-patient variability represent the unexplained random variability. No estimate for between- or within-patient variability does not mean that there is no variability in the concerning parameter, but that the data do not contain sufficient information to allow reliable estimation of the variability.

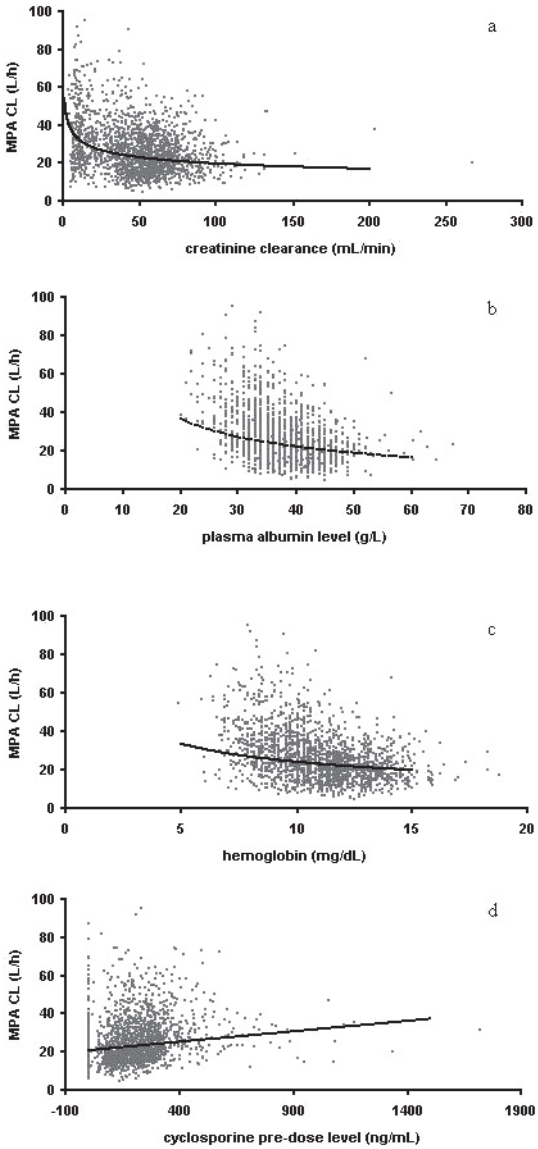


Figure 2 Correlations between MPA clearance (CL) and renal function (creatinine clearance calculated according to Cockcroft and Gault) (a), plasma albumin level (b), haemoglobin (c) and cyclosporine pre-dose level (d). The solid lines represent the correlation estimated by the final population pharmacokinetic model.

Furthermore, absorption half-life was found to be significantly longer with a lower cyclosporine dose: 0.15 h with a cyclosporine dose of 500 mg and 0.26 h without the use of cyclosporine ($p<0.001$), indicating that cyclosporine increased the rate of MPA absorption from the gut. Patients using antacids had a 37% higher central volume of distribution than patients who did not use these agents.

The parameter estimates of the final model are summarised in table 4. The identified relationships between patient factors and pharmacokinetic parameters after the multivariate analysis (table 3) explained both between- and within-patient variability in the pharmacokinetics of MPA. 18% of the between-patient variability in clearance and 38% of the within-patient variability in clearance was explained. For absorption half-life, 35% between- and 15% within-patient variability could be explained. For central volume of distribution, 39% between- and 20% within-patient variability was explained.

Finally, 42% between- and 47% within-patient variability was explained for intercompartmental clearance. The magnitude of the effect of renal function on MPA clearance and of albumin level on MPA clearance could be very different per individual as illustrated by coefficients of variation for the between-patient variability in these relationships of 66% and 112% respectively ($p<0.001$). This indicates that a change of renal function or albumin level may have a significant impact on MPA exposure in one patient, while in another patient the effect may be considerably less.

Effects of cyclosporine exposure, DGF, race and diabetes mellitus

To further illustrate the influence of the use of cyclosporine on MPA exposure, the course of dose-normalised MPA AUC_{0-12} over time after transplantation was compared between patients who had cyclosporine as concomitant immunosuppressive therapy (n=144 on day 0-4 posttransplant) and patients who used an immunosuppressive regimen without cyclosporine (n=102 on day 0-4 posttransplant) (figure 3). Part of this latter group was concurrently treated with sirolimus (n=30). Patients who were exposed to cyclosporine exhibited lower median dose normalised MPA AUC_{0-12}-values than patients not exposed to cyclosporine during the whole study period with the exception of the first week. At months 1, 3, and 6 and at year 1 after transplantation, patients who used cyclosporine had a median dose normalised MPA AUC_{0-12} of respectively 36, 45, 52 and 56 mg*h/L and patients without cyclosporine exposure had a median MPA AUC_{0-12} of 65, 58, 77 and 72 mg*h/L. Of note, these values also show that MPA exposure increased with time posttransplant.

In the univariate analysis, patients with DGF had a significantly lower median MPA AUC_{0-12} compared to those with immediate graft function during the first four days after transplantation (23 versus 33 mg*h/L respectively, $p<0.001$, figure 4). However, in the multivariate analysis DGF was no longer a significantly correlated with clearance (table 3), because renal function, as the more broadly defined variable, could explain the lower MPA exposure in patients with DGF: median C&G was 10 mL/min in patients with DGF versus 23 mL/min in patients with immediate graft function during the first four days after transplantation ($p<0.001$). Thereafter, with recovering renal function in patients with DGF (21 mL/min in week 2) the difference in MPA AUC_{0-12} between patients with or without DGF decreased: 27 versus 33 mg*h/L during day 5 to 8, and 31 versus 33 mg*h/L during week 2.

Black renal transplant patients exhibited lower median dose normalised MPA AUC_{0-12}-values during the first month after transplantation compared to Caucasian patients. AUC_{0-12}-values on day 0-4, day 5-8, week 2 and month 1 were 30, 25, 25 and 30 mg*h/L for Blacks and 32, 32, 33 and 38 mg*h/L for Caucasians. Race however was not a significantly correlated with clearance in the multivariate analysis (table 3). The difference may therefore be the result of a lower median renal function in Blacks during the same occasions (10, 16, 25 and 52 mL/min) compared to Caucasians (21, 29, 38 and 55 mL/min). The level of renal function over time between both races, however, was not statistically significant ($p=0.18$). This may be due to the small number of Blacks per occasion (n=17, 8, 6, and 7 respectively), resulting in insufficient power to find a statistically significant difference.

In this study, diabetic patients had a small but significantly increased T_{max} (calculated according to reference [38]) compared to non-diabetic patients during the first half year after transplantation. For example, median T_{max} in diabetic renal transplant patients at one month posttransplant was 1.1 h and 0.8 h in non-diabetic patients ($p=0.045$).

Figure 3 Course of dose-normalised MPA area-under-the-curve (AUC_{0-12}) over time after transplantation for patients who had cyclosporine (CsA) as concomitant immunosuppressive therapy (n=144 on day 0-4 posttransplant) (open Box-Whisker plots) and for patients who used an immunosuppressive regimen without CsA (n=102 on day 0-4 posttransplant) (closed Box-Whisker plots). The box indicates the upper and lower quartiles and the central line represents the median. The whiskers represent the 2.5% and the 97.5% values. The dotted lines represent the adopted therapeutic window for MPA AUC_{0-12} values of 30 to 60 mg*h/L [9]. Exposure was significantly different between groups with $p<0.05$ on month 1 and 6 and year 1. Exposure was significantly different between groups with $p<0.01$ on month 3.

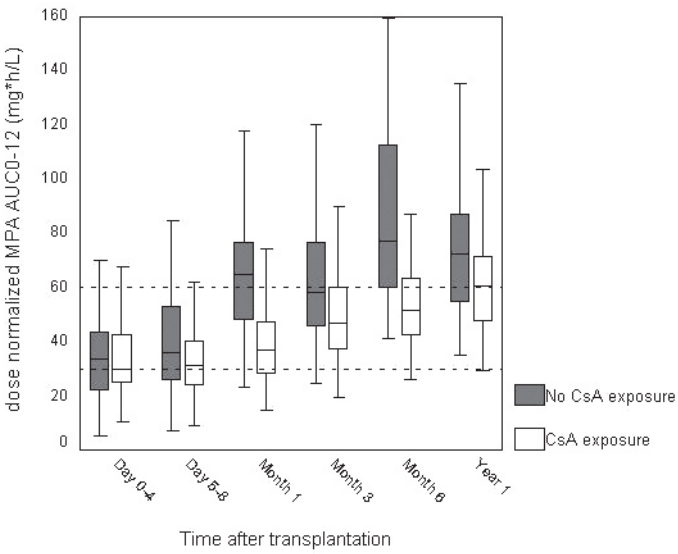
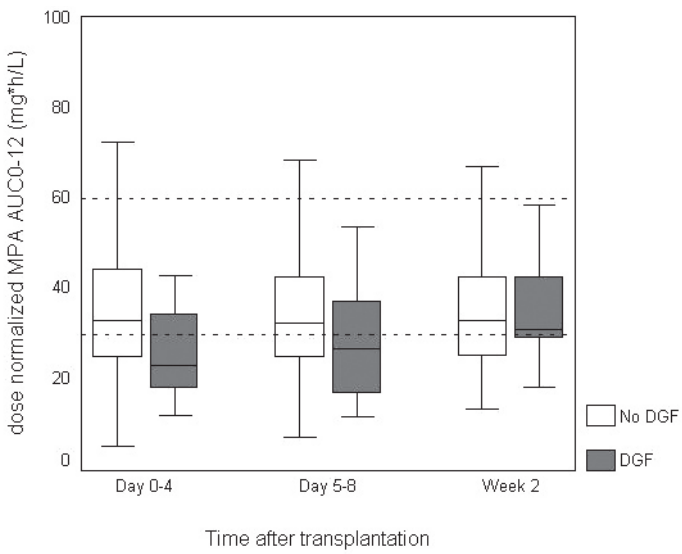


Figure 4 Course of dose-normalised MPA area-under-the-curve (AUC_{0-12}) over the first two weeks after transplantation for patients with immediate graft function (n=212 on day 0-4 posttransplant) (open Box-Whisker plots) and for patients with delayed graft function (DGF, n=34 on day 0-4 posttransplant) (closed Box-Whisker plots). The box indicates the upper and lower quartiles and the central line represents the median. The whiskers represent the 2.5% and the 97.5% values. The dotted lines represent the adopted therapeutic window for MPA AUC_{0-12} values of 30 to 60 mg*h/L [9]. Exposure was significantly different between groups with $p<0.05$ on day 0-4.



DISCUSSION

During standard dose MMF therapy, the MPA exposure has been reported to vary 10-fold between patients, resulting in a wider range of MPA AUC values than the adopted AUC range of the therapeutic window [4]. This suggests that dose individualisation may improve outcome. Several studies investigated the determinants of the variability in MPA concentrations, but conflicting results have been obtained [3,5,9-11,13-25]. To explore which factors can explain variability in the pharmacokinetics of MPA, a powerful population pharmacokinetic meta-analysis was performed using data from 468 renal transplant recipients. Eight variables were identified which significantly influenced the pharmacokinetics of MPA (table 3). With these eight variables, 18% to 42% of the between-patient variability and 15% to 47% of the within-patient variability can be explained in the different pharmacokinetic parameters.

Renal function was an important determinant of MPA clearance. MPA clearance decreased with improving renal function. This correlation could explain 35% of within-patient variability, meaning that recovering renal function can explain an important part of the well-known increase of MPA exposure within a patient over time [7]. The relationship between renal function and MPA clearance also explained why patients with DGF had a higher CL and consequently a lower MPA $AUC_{0\text{-}12}$ in the first days after transplantation compared to patients with immediate graft function (figure 4) [9,39]. Patients with DGF had lower MPA exposure as a result of a significantly lower renal function during that period compared to patients without DGF.

A similar effect may apply to race. Black patients showed a trend towards lower dose normalised MPA $AUC_{0\text{-}12}$ compared to Caucasian patients in the first month after transplantation. Like DGF, this difference may be explained by a lower renal function in Blacks, without an additive effect attributable to race. Although speculative, a possible difference in renal function between races and the resulting effect on MPA exposure might have contributed to the observation in a previous study that Black patients only benefited from MMF over azathioprine with doses of 1.5 g twice daily, instead of the standard dose of 1 g twice daily [9,25].

The influence of renal function on MPA clearance was not found in every study investigating the pharmacokinetics of MPA [15,16,18,19]. This is explained by the fact that renal function only appears to have a clinically relevant effect on MPA clearance if renal function is below 25 mL/min (figure 2a). Changes in renal function above the 25 mL/min threshold have a small impact on MPA clearance: an improvement of renal function from 65 to 110 mL/min induces a modest decrease of MPA clearance from 22 to 19 L/h (figure 2a). Studies with low proportions of patients with delayed or impaired graft function postoperatively may have been underpowered to demonstrate the influence of renal function on MPA clearance.

Acidosis, uremia and accumulation of MPAG, all associated with impaired renal function, will decrease MPA binding to albumin [9]. As MPA is supposed to be a restrictively cleared drug, an increased free fraction leads to an increase of the amount of MPA available for glucuronidation and hence to a higher MPA clearance [9].

The relationship with plasma albumin level and MPA clearance is also explained through MPA free fraction. When albumin levels increase, MPA free fraction may become smaller and consequently MPA clearance may decrease. The effect that increasing haemoglobin caused a decrease in MPA clearance, which has not been found earlier, might also be explained with the same hypothesis. This suggests that MPA does not only bind to albumin, but also to haemoglobin or red blood cells. Unfortunately, free MPA concentrations were not available in this study to test this hypothesis.

Despite having identified the significant influence of renal function and plasma albumin level on MPA clearance, adjustments of MMF dose cannot be recommended purely based on these factors. The reason is that large between-patient variability was estimated in the effect that renal function and albumin level had on MPA clearance (66 and 112% respectively). This means that the same change in renal function or albumin level in one patient may result in a clinically relevant change of MPA clearance, while in another patient hardly any effect will be present. Consequently, a change in renal function or albumin level is not in itself an indication for dose adjustment, but is merely an indication for therapeutic drug monitoring to check whether the MMF dose needs to be adjusted in order to get or keep MPA exposure on target. Another reason may be that despite lower total MPA exposure, free MPA concentrations may be unaltered or even elevated in situations of impaired renal function or low albumin levels [9,40]. Because free MPA is regarded as the pharmacologically active moiety [41], MMF dose adjustments would not be indicated then. This issue warrants further research before MMF dose can be based on renal function and albumin level.

The observation that MPA clearance is influenced by cyclosporine pre-dose level can be explained by cyclosporine mediated inhibition of the multidrug resistance protein 2 through which the enterohepatic recirculation of MPA can be disrupted [10]. The result is that patients concurrently treated with cyclosporine had lower MPA exposure than patients not receiving cyclosporine during the first year after renal transplantation (figure 3). This observation is in accordance with observations from other studies in which patients concurrently treated with sirolimus [42] or tacrolimus [21] had higher MPA exposure than cyclosporine-treated patients. Furthermore, figure 3 shows that half of the patients who were concurrently treated with cyclosporine had MPA exposure below the recommended target window in the first week after transplantation. Because optimal MPA exposure early after transplantation is associated with a lower incidence of acute rejection [43], outcome in patients in whom MMF is combined with cyclosporine may be improved with 1500 mg MMF twice daily instead of the currently recommended 1000 mg twice daily in the immediate posttransplant phase.

The result from a previous study that the tapering of corticosteroids leads to an increase of MPA concentrations could not be confirmed [22]. A positive correlation between the corticosteroid dose and MPA clearance could be identified during the univariate analysis, but this relationship lost its significance in the multivariate analysis. This indicates that the corticosteroid dose is a confounding factor for the relationships between the patient factors and MPA clearance in the final model.

A previous study did not show an effect of diabetes mellitus on MPA $AUC_{0\text{-}12}$ [23]. Another study found an increased T_{max}, but only seven diabetic patients were included [24]. This study confirms a slightly increased T_{max} in diabetic renal transplant recipients. The increased T_{max} may be related to gastroparesis, which is present in many diabetic patients [44], but does not have a clinically relevant impact on MPA exposure.

Figure 3 shows that dose normalised MPA $AUC_{0\text{-}12}$ increases over time after renal transplantation as a result of decreasing clearance. Given the identified relationships between MPA clearance and renal function, haemoglobin, albumin level and cyclosporine pre-dose level, this is in part caused by dynamic changes in these variables. The increase in exposure in the group without cyclosporine exposure mainly occurs in the first month after transplantation and may thus be caused by improving renal function, increasing albumin level and climbing haemoglobin (figure 3). Increasing MPA exposure later after transplantation may be, in part, the result of a decrease in cyclosporine pre-dose levels (table 2). This is illustrated in the cyclosporine group in figure 3 where median renal function and albumin level were quite stable between month 1 and year 1 (median renal function increased from 52 to 64 mL/min and median albumin level increased from 37 to 39 g/L), while median cyclosporine pre-dose level decreased

from 237 to 155 ng/mL during that period. As a result of the gradual increase in MPA exposure, a subset of patients will be above the target window with standard MMF doses of 1000 mg twice daily after 6 to 12 months after transplantation. This is most likely in patients who are no longer treated with cyclosporine and who have good renal function, albumin level and haemoglobin [42]. The increased MPA exposure may be very welcome in regimens in which cyclosporine is tapered or stopped, and in patients who tolerate such levels without toxicity dose reductions may not be necessary. It is also important that the recommended target window [8] is based on a combination of MMF with a calcineurin inhibitor, and other target values may apply for other combinations [45].

CONCLUSION

In summary, with a population pharmacokinetic model relationships have been identified between patient factors and pharmacokinetic parameters, thus explaining variability in MPA pharmacokinetics. Exposure to MPA is significantly influenced by renal function, albumin level, haemoglobin and cyclosporine pre-dose level. These variables may prove to be useful for more effective therapeutic drug monitoring and MMF dosing, but this warrants further prospective research. Differences in MPA exposure between patients with or without DGF or between patients of different races, are likely to be caused by the effect of renal function on MPA exposure. Diabetes mellitus, the use of gastric pH modulators other than antacids, corticosteroids, antibiotics and antiviral agents do not have an effect on MPA exposure.

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Chapter 2.3

Mycophenolic Acid in Diabetic Renal Transplant Recipients: Pharmacokinetics and Application of a Limited Sampling Strategy

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ABSTRACT

Limited sampling strategies offer support to optimise therapeutic drug monitoring of mycophenolic acid (MPA). Their use however may be limited by several patient factors, including co-morbidity. In this study the pharmacokinetics of MPA in diabetic and non-diabetic renal transplant recipients were compared and it was evaluated whether a developed limited sampling strategy validated for non-diabetic patients can also be used in diabetic patients.

The pharmacokinetics of MPA were analysed in 136 renal transplant patients, among whom seven diabetics, on day 7 and 11 after transplantation. All patients received cyclosporine and corticosteroids as maintenance immunosuppressive therapy. A limited sampling strategy (AUC [mg*h/L] = 7.182 + $4.607xC_0$ + $0.998xC_{0.67}$ + $2.149xC_2$) was developed and validated for non-diabetic patients and was subsequently tested for its usefulness in diabetic patients.

Diabetic renal transplant patients did not have significantly different dose normalised MPA area under concentration time curve (AUC), MPA clearance or MPA maximum concentration (C_{max}). However in diabetic patients T_{max} (time of C_{max}, 1.59 h) was higher than for non-diabetic patients (0.67 h) on day 11 (p=0.04).

The developed and validated limited sampling strategy performed acceptable, estimating MPA AUC in non-diabetic patients with a mean bias of 0.2 mg*h/L (95% confidence interval from –1.3 to 1.6 mg*h/L). Applying the limited sampling strategy in diabetic patients revealed a mean bias of –1.5 (-5.7, 2.7 mg*h/L).

In conclusion, although diabetic renal transplant patients exhibit increased T_{max}, this does not affect the accuracy of the limited sampling strategy.

INTRODUCTION

Mycophenolic acid (MPA), the active component of the pro-drug mycophenolate mofetil (MMF), is an immunosuppressant effectively used for the prevention of acute rejection after renal transplantation [1]. Recent studies have demonstrated a relationship between the area under the plasma concentration versus time curve (AUC) of MPA and the risk for acute graft rejection and side effects [2,3]. Nevertheless, there is still debate on the value of therapeutic drug monitoring (TDM) of MPA [4,5]. Part of the discussion focuses on which pharmacokinetic parameter should be employed for optimisation of MPA therapy. The AUC_{0-12} of MPA correlates fairly well with clinical outcome, but its determination is inconvenient and time-consuming as a tool for TDM due to the fact that serial blood samples have to be collected at pre-defined time points. Trough concentrations (C_{min}) are less inconvenient, but do not correlate as good with the risk of acute rejection and side effects as the AUC_{0-12} of MPA [2]. According to several studies, limited sampling strategies may offer a solution, since MPA AUC can be estimated on the basis of a limited number of blood samples, usually collected within two to six hours after oral intake of the drug [6-10]. Food intake, co-medication and co-morbidity however may complicate the use of limited sampling strategies in TDM of MPA [5,6,11]. For example, a change in MPA absorption rate is thought to hinder an accurate estimation of full AUC from limited sampling strategies [12].

Particular disease-states may alter pharmacokinetics such that a standard limited sampling strategy is not appropriate. Altered pharmacokinetics, including changes in absorption, distribution and elimination, has been described for several drugs in diabetic patients [13]. If this is also the case for MPA, the estimated MPA AUC derived from a limited sampling strategy may be biased in diabetic renal transplant recipients.

In this study we assessed the influence of diabetes on the pharmacokinetics of MPA and determined whether or not limited sampling strategies validated for non-diabetic patients can also be used in diabetic patients.

PATIENTS AND METHODS

Patients

Concentration-time samples from one hundred thirty-six renal transplant patients who participated in a Randomised Concentration Controlled Trial (RCCT) were analysed retrospectively. For a detailed description of the methods of the RCCT, the reader is referred to the literature [2,10]. Briefly, the goal of the RCCT was to investigate the relationship between exposure to MPA (AUC, C_{max}, C_{min}) and clinical outcome (risk for acute rejection, side effects). Patients were randomly assigned to three AUC target groups: low (16.1 mg*h/L), intermediate (32.2 mg*h/L) or high (60.6 mg*h/L) AUC. Starting MMF doses were 450 mg bid for the low, 950 mg bid for the intermediate and 1700 mg bid for the high target AUC group. Pharmacokinetic assessment based on eight sampling time points started at day three after transplantation and from that point on MMF doses were adjusted according to MPA AUC assessment on eight further occasions in 20 weeks. All patients received cyclosporine and corticosteroids as maintenance immunosuppressive therapy.

Pharmacokinetic analysis

Plasma samples for description of 12-h concentration-time profiles for the present analysis were collected on day seven and day eleven after transplantation. Samples were collected before oral administration of MMF, at 0.33, 0.67, 1.25, 2, 6, 8 and 12 hours after administration. Patients were required to fast overnight prior to dosing and for the first two hours of the profile. Samples were analysed using a validated HPLC method [14]. The concentration-time data were analysed using WinNonlin version 3.1 (Pharsight Corporation, Mountain View, CA, USA). A noncompartmental model with extravascular input for plasma data was used to obtain estimates for MPA maximum concentration (C_{max}), time of maximum concentration (T_{max}) and AUC over the period 0 to 12 hour. The AUC_{0-12} was calculated by using the logarithmic trapezoidal rule. Apparent oral clearance of MPA (Cl) was calculated by dividing the MMF dose by the AUC. Diabetic and non-diabetic patients were compared using MPA AUC normalised to 1 gram of MMF, because of the large dose ranges in the three target AUC groups. The dose normalised AUC_{0-12} was calculated using formula 1:

Dose normalised AUC = measured AUC x (1000 mg MMF / actual MMF dose in mg) (Eq. 1)

Limited sampling strategy

A limited sampling strategy was developed based on data from the RCCT collected on days 7 and 11 after transplantation from the three target AUC groups. Data were split into two data sets. The first was an index data set, which was used to develop the limited sampling strategy. It contained 60 non-diabetic renal transplant recipients, randomly selected from the three target AUC groups (20 per group). MPA concentrations at each sampling time point were correlated with the measured MPA AUC_{0-12} using linear regression analysis. Those MPA concentrations at sampling time points that showed the best correlation were combined to improve the correlation in a multiple stepwise linear regression analysis, with MPA AUC_{0-12} as independent variable and the MPA concentrations at the different sample time points as explanatory variables. For practical reasons a limited sampling strategy could consist of a

maximum of three sampling time points with the latest sample being collected maximally 2 hours after administration of MMF. The second data set was a validation data set, which included the remaining non-diabetic and 7 diabetic renal transplant recipients, used to correlate the $AUC_{0\text{-}12}$ estimated with the developed limited sampling strategy with the measured MPA $AUC_{0\text{-}12}$, thus verifying the performance of the developed strategy. This data set was also used to compare the usefulness of the limited sampling strategy between diabetic and non-diabetic renal transplant patients.

To assess the agreement between the measured and the estimated MPA AUC, the correlation coefficient, r^2, was calculated. Also, the mean prediction error (MPE) or bias and the root mean squared prediction error (RMSE) or precision of the comparison of the estimated AUC with the measured AUC were obtained using formula 2 and 3 respectively [15].

$$MPE = \frac{\sum_{i=1}^{N} (pe_i)}{N} \qquad \text{(Eq. 2)}$$

$$RMSE = \sqrt{\left(\frac{\sum_{i=1}^{N} (pe_i^2)}{N}\right)} \qquad \text{(Eq. 3)}$$

N represents the number of pairs of estimated and measured AUC and pe_i is the difference between the estimated and the measured AUC. Bias and precision were visualised by plotting the average AUC resulting from the abbreviated and the full profile as described by Bland and Altman [16].

Statistics

Statistical tests were performed with the software package SPSS 10.1 for Windows (SPSS Inc. Chicago, IL, USA). Patient characteristics are presented as mean with standard deviation and range. Pharmacokinetic data are expressed as median and range, since data were not normally distributed. To facilitate comparison with pharmacokinetic data from literature also mean and standard deviation are provided. Bias and precision of the comparison between measured and estimated MPA AUC are expressed as mean and their 95% confidence intervals (95% CI). The Mann-Whitney U test was used to test for statistical differences. A p-value of 0.05 was considered statistically significant. Since only seven of the one hundred thirty-six renal transplant patients suffered from diabetes mellitus a power analysis was done to calculate the power with which differences between diabetic and non-diabetic patients can be detected. This was done according to the method with unequal sample sizes described by Altman [17].

Results

In total, data from 136 renal transplant patients, 129 non-diabetics and 7 diabetics, were studied during the pharmacokinetic analysis. On day 7 after transplantation data were available from 128 non-diabetic and from 7 diabetic patients, as on day 11, data from 125 non-diabetic and 6 diabetic patients were available. MMF doses ranged from 400 mg bid to 2200 mg bid in non-diabetic renal transplant recipients and from 450 mg bid to 2000 mg bid for diabetic patients. The results from the pharmacokinetic analysis are summarised in table 1. The median Cl for non-diabetic renal transplant patients on day 7 was 42 L/h and 43 L/h on day 11. Median Cl for diabetic patients was 38 L/h on day 7 and 36 L/h on day 11. The Mann-Whitney U test showed that Cl between the two subgroups on both days was not different (p=0.19 on day 7 and p=0.43 on day 11). There was also no significant difference in the median dose normalised $AUC_{0\text{-}12}$ between patients with and without diabetes: 26 mg*h/L versus 24 mg*h/L, p=0.19 on day 7 and 28 mg*h/L versus 23 mg*h/L, p=0.43 on day 11. The observed median C_{max} from diabetic patients was 6.7 mg/L on day 7 and 9.3 mg/L on day 11 and not significantly different from the median C_{max} observed in patients without diabetes (9.0 mg/L on day 7 with p=0.99 and 10.1 mg/L on day 11, p=0.77). A non-significant trend was observed in increased median T_{max} for diabetics compared to non-diabetics on day 7 (1.25 h respectively 0.67 h, p=0.13). This trend became statistically significant on day 11 (1.59 h versus 0.67 h for non-diabetic patients, p=0.04 (figure 1)). The power analysis revealed that the power with which this significant difference was identified was approximately 80%.

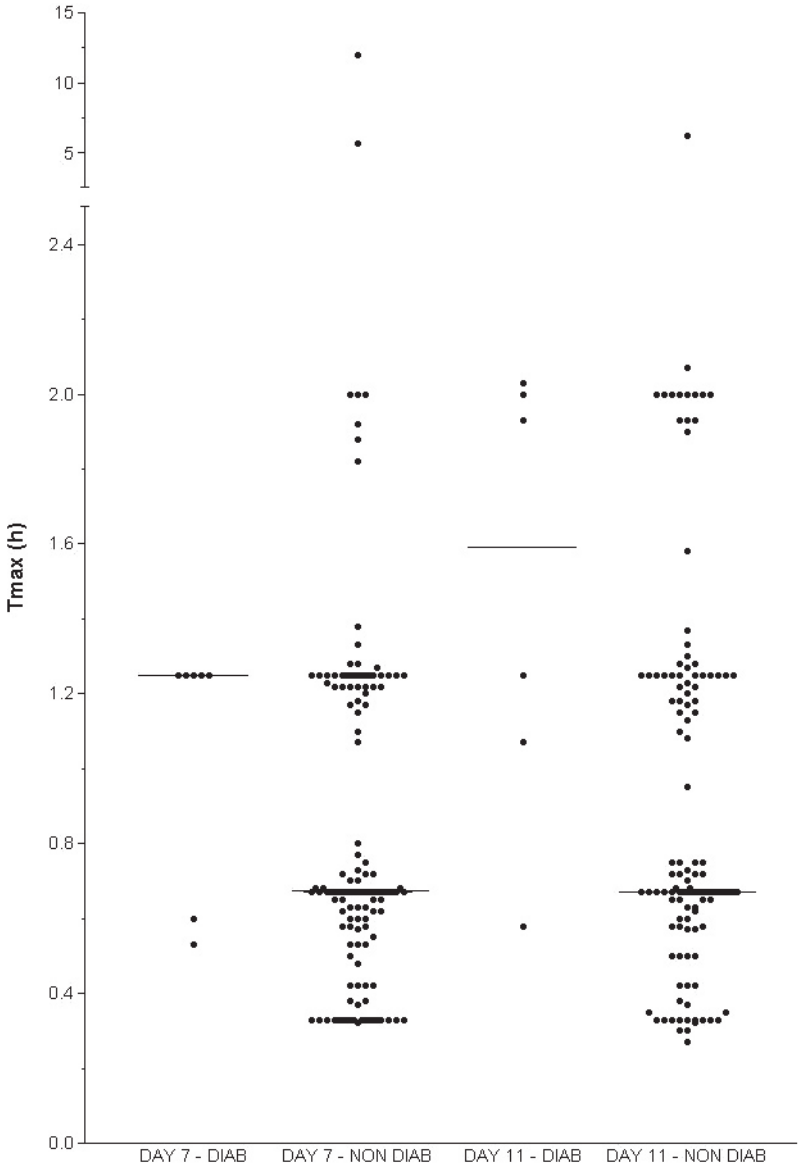
Table 1 Pharmacokinetic parameters of MPA for diabetic patients versus non-diabetic patients on day 7 and day 11 after renal transplantation.

	Day 7			**Day 11**		
	Diabetic patients n=7	**Non-diabetic patients n=128**	**p**	**Diabetic patients n=6**	**Non-diabetic patients n=125**	**p**
T_{max} (h)	1.25 (0.53-1.25) 1.05±0.33	0.67 (0.32-12.00) 0.94±1.15	0.13	1.59 (0.58-2.03) 1.48±0.60	0.67 (0.27-6.17) 0.94±0.67	0.04
C_{max} (mg/L)	6.7 (4.1-21.0) 10.5±6.4	9.0 (0.5-58.4) 11.7±9.9	0.99	9.3 (3.6-21.4) 10.6±6.1	10.1 (1.3-86.9) 13.0±11.0	0.77
$AUC_{0\text{-}12}$ (mg*h/L)	29 (14-51) 31±15	25 (5-106) 28±17	0.57	28 (13-39) 27±12	28 (8-69) 30±16	0.78
*Dose normalised AUC (mg*h/L)	26 (20-72) 34±18	24 (6-89) 26±14	0.19	28 (18-45) 28±10	23 (6-71) 25±12	0.43
Cl (L/h)	38 (14-51) 35±12	42 (11-180) 50±27	0.19	36 (22-57) 39±13	43 (14-181) 49±27	0.43

Values expressed as median (range) as well as mean±standard deviation. AUC = mycophenolic acid area under the concentration time curve, C_{max} = maximum concentration, T_{max} = time of maximum concentration, MPA CL = mycophenolic acid apparent oral clearance.

*Dose normalised AUC's are normalised to 1 gram of mycophenolate mofetil.

Figure 1 Time of maximum MPA concentration (T_{max}) for diabetic (DIAB) and non-diabetic (NON DIAB) patients for both sampling days. Horizontal lines represent the median T_{max}.



Limited sampling strategy

The limited sampling strategy analysis studied 133 renal transplant patients, since three non-diabetic patients were excluded for reasons of insufficient concentration-time data for AUC estimation through a limited sampling profile. The demographics for the remaining population are summarised in table 2. The index data set contained 60 non-diabetic patients from whom 118 concentration-time profiles were available. The validation data set consisted of the 7 diabetic and 66 non-diabetic patients from whom 13 and 126 concentration-time profiles were available respectively. Patients with diabetes were not significantly different from patients without diabetes with regard to age, weight, MMF dose, albumin concentration and serum creatinine. MPA concentrations at each sample time point in the index data set, until two hours after oral intake of MMF, were correlated with the measured MPA $AUC_{0\text{-}12}$ in a linear regression analysis. Correlation coefficients (r^2) for the separate sampling time points were: 0.45, 0.35, 0.58, 0.59 and 0.42 at predose, 0.33, 0.67, 1.25 and 2 hours after administration respectively. The p-value for all correlations was smaller than 0.0001. The multiple stepwise regression analysis revealed that the best estimate of the measured MPA $AUC_{0\text{-}12}$ was derived by the combination of the sampling time points at predose, 0.67 hours and 2 hours after oral intake of MMF. The correlation coefficient was 0.73 and mean bias was 0.0 mg*h/L (table 3). The accompanying algorithm is described by formula 4.

$$AUC\ [mg*h/L] = 7.182 + 4.607 \times C_0 + 0.998 \times C_{0.67} + 2.149 \times C_2 \qquad \text{(Eq. 4)}$$

To test the agreement between the measured MPA $AUC_{0\text{-}12}$ and the estimated AUC, the developed limited sampling strategy was applied to the validation data set without the diabetic renal transplant recipients. The validation of the strategy yielded a r^2 of 0.67 and a non-significant mean bias of 0.2 mg*h/L (95% CI: –1.3, 1.6), table 3. Precision was 8.1 mg*h/L. The performance of the limited sampling strategy was further characterised by a percentage of 62% of estimated AUC's that fell within 75-125% of the measured MPA AUC. When the developed limited sampling strategy was applied to the diabetic subgroup in the validation data set, bias was –1.5 mg*h/L, which was not significantly different from 0 mg*h/L (95% CI: -5.7, 2.7 mg*h/L). Precision in diabetic patients was 6.9 mg*h/L and the correlation coefficient was 0.75. 62% of the estimated AUC's in the diabetic subgroup fell within 75-125% range of the measured MPA AUC. The predictive performance of the limited sampling strategy for diabetics and non-diabetics in the validation data set is illustrated in figures 2 and 3 (Bland-Altman plots) and is summarised in table 3. The power with which a small difference of 5 mg*h/L between diabetic and non-diabetic patients in MPA AUC estimated from the limited sampling strategy can be detected within the present study was 50%. Larger differences, such as 6.5 or 10 mg*h/L could be detected with a power of 80% and 98% respectively.

Table 2 Patient demographics

		Index data set	Validation data set		
			Non-diabetic patients	Diabetic patients	*p
Number of subjects		60	66	7	
Target AUC group	Low (16.1 mg*h/L)	20	20	3	
	Intermediate (32.2 mg*h/L)	20	23	2	
	High (60.6 mg*h/L)	20	23	2	
Number of profiles		118	126	13	
Gender					
	Male	35	44	4	
	Female	25	22	3	
MMF dose range (mg bid)		1152±606 (550-2200)	1281±645 (300-2200)	1092±642 (450-2200)	0.31
Age (years)		48±12 (19-69)	48±12 (20-70)	52±6 (43-61)	0.23
Weight (kg)		73±14 (36-104)	68±12 (42-99)	66±12 (52-83)	0.64
Albumin (g/L)					
	Sample day 1	35±4 (26-44)	34±4 (24-44)	33±4 (26-38)	0.92
	Sample day 2	35±5 (26-46)	35±4 (27-44)	34±5 (26-39)	0.97
Serum creatinine (µmol/L)					
	Sample day 1	291±266 (70-1153)	271±224 (70-1091)	158±73 (79-299)	0.23
	Sample day 2	251±226 (79-1038)	227±196 (53-1118)	132±48 (79-194)	0.14

Values are expressed as mean with standard deviation (range).
* p-value for the comparison between diabetic patients and non-diabetic patients in the validation data set.

Table 3 Predictive performance of the limited sampling strategy.

	Index data set	Validation data set	
	118 profiles	Diabetic patients 13 profiles	Non-diabetic patients 126 profiles
Bias (mg*h/L)	0.0 (-1.7,1.7)	-1.5 (-5.7,2.7)	0.2 (-1.3,1.6)
Precision (mg*h/L)	9.3 (6.7,11.3)	6.9 (2.9,9.2)	8.1 (6.5,9.4)
r^2	0.73 (0.67,0.82)	0.75 (0.44,0.92)	0.67 (0.61,0.77)

Bias, precision and coefficient of determination and their 95% confidence intervals for the comparison between the limited sampling strategy (AUC [mg*h/L] = $7.182 + 4.607xC_0 + 0.998xC_{0.67} + 2.149xC_2$) and full MPA AUC.

Figure 2 Bland-Altman plot for the agreement between measured (full) MPA $AUC_{0\text{-}12}$ and estimated (est) MPA $AUC_{0\text{-}12}$ for diabetic patients in the validation data set, both sampling days added. The line represents the mean bias, dotted lines are plus and minus two times the standard deviation of the mean.

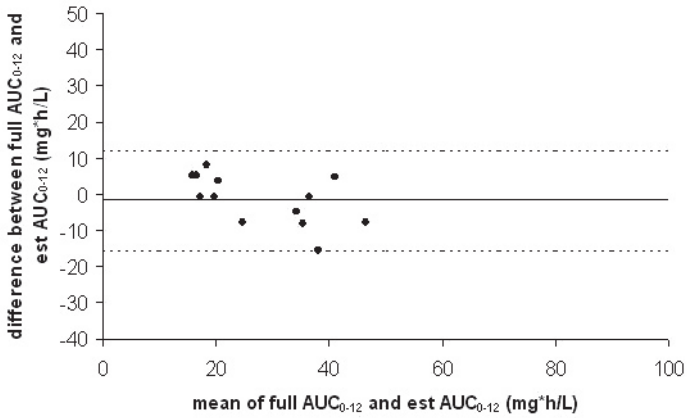
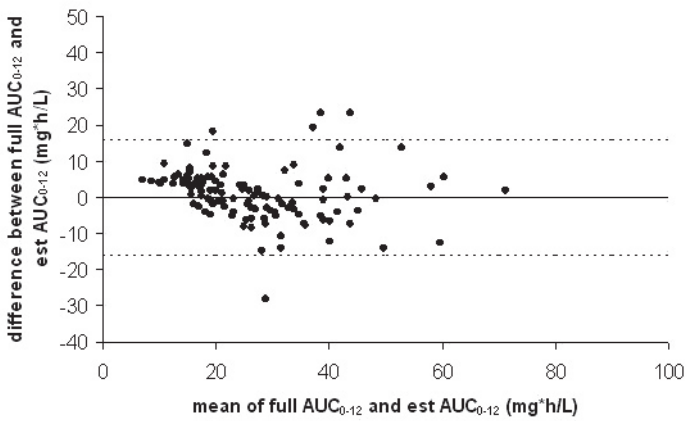


Figure 3 Bland-Altman plot for the agreement between measured (full) MPA $AUC_{0\text{-}12}$ and estimated (est) MPA $AUC_{0\text{-}12}$ for non-diabetic patients in the validation data set, both sampling days added. The line represents the mean bias, dotted lines are plus and minus two times the standard deviation of the mean.



DISCUSSION

This study shows no significant differences between diabetic and non-diabetic renal transplant recipients in dose normalised AUC_{0-12}, Cl and C_{max}. However, absorption appears to be delayed with a trend towards increased T_{max} for diabetic patients on day 7 post-transplant, which is significant on day 11. The prevalence of diabetes in a population of renal transplant patients is estimated to be two- to threefold higher as compared to the general population [18]. In diabetic patients gastroparesis is a well-described feature, with an estimated prevalence of 27-58% in type I diabetes and about 30% in type II diabetes [19]. Gastroparesis may cause slower absorption of MPA, which may lead to an increased T_{max} in the concentration time profile [12]. This might explain the increased T_{max} observed in the present study.

The developed and validated limited sampling strategy was based on MPA concentrations at predose, and 0.67 and 2 hours after oral intake of MMF. It is known that when the evaluation of the performance of a strategy is based on the same data as it was derived from, biased results could be produced [20]. Consequently, the validation of the present strategy was done on a separate data set. The validation of the limited sampling strategy was mainly based on calculation of mean bias and mean precision, rather than the correlation coefficient. This was done because Bland and Altman as well as Sheiner and Beal showed that evaluating a limited sampling strategy only by means of a r^2 provides biased results [15,16]. The mean bias was 0.2 mg*h/L and mean precision was 8.1 mg*h/L, which indicates that the developed limited sampling strategy has acceptable predictive performance.

Further validation of the developed strategy showed a correlation coefficient of 0.67. This resulting r^2 for the non-diabetic subgroup in the validation data set seems low compared to limited sampling strategies described in literature [6-8,21,22]. However, most limited sampling strategies for MPA reported in literature tested their algorithm on the same data as it was developed upon, instead of validating the strategy on a separate data set [8,21,22]. This does not only mean that the correlation coefficient for the limited sampling strategy developed in this study is not directly comparable with most of the reported values from literature, but also that values for r^2 reported in literature might be biased [20].

Limited sampling strategies that do not contain samples after 2 hours postdose are thought to predict AUC less accurately when T_{max} is increased [6,12]. An increased T_{max} may cause an underestimation of AUC from limited sampling strategies, especially in cases where the actual T_{max} falls outside the sampling time period. In the present study T_{max} is increased in diabetic renal transplant recipients, but it still falls within the 2-hour sampling period of the limited sampling strategy. As a consequence, the comparison between the measured AUC_{0-12} and the AUC calculated from the limited sampling strategy showed a non-significant mean bias of -1.5 mg*h/L with 95% CI from –5.7 to 2.7 mg*h/L. Although the mean bias shows that the measured AUC_{0-12} is slightly underestimated, its magnitude is small and comparable with the mean bias observed in non-diabetic patients (figures 2 and 3).

Due to the small number of concentration-time profiles from diabetic renal transplant patients (n=13), a power analysis revealed that this study lacks power concerning the detection of small differences (6.5 mg*h/L and smaller) in the estimated MPA AUC between diabetic and non-diabetic patients. However, larger differences (6.5 mg*h/L and larger), which are more likely to affect clinical decision making given the wide recommended therapeutic MPA AUC range of 30-60 mg*h/L [4], are detected with reasonable power. Since no apparent difference was observed, this leads to the conclusion that the limited sampling strategy developed and validated for non-diabetic patients, does not produce clinically relevant biased estimates of the MPA AUC_{0-12} in diabetic patients. Still, the results must be interpreted with caution and further studies with larger subgroups of diabetic renal transplant patients are necessary to confirm the results of the present study.

CONCLUSION

Renal transplant recipients with diabetes do not present statistically significantly different MPA pharmacokinetics relative to renal transplant patients without diabetes with regard to dose normalised MPA $AUC_{0\text{-}12}$, MPA CI and C_{max}. However, a trend on day 7 towards increased T_{max}, which is significant on day 11, is present. This increased T_{max} in diabetic patients does not affect the clinical utility of the developed and validated limited sampling strategy in this subgroup.

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Chapter 2.4

Time-dependent Clearance of Mycophenolic Acid in Renal Transplant Recipients

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ABSTRACT

Aim: Pharmacokinetic studies of the immunosuppressive compound mycophenolic acid (MPA) have shown a structural decrease in clearance (CL) over time after renal transplantation. The aim of this study was to characterise the time-dependent CL of MPA by means of a population pharmacokinetic meta-analysis, and to test whether the time-dependency of CL can be described by covariate effects.

Methods: 1894 MPA concentration-time profiles from 468 renal transplant patients (range: 1-9 profiles per patient) were analysed retrospectively with nonlinear mixed effect modeling. Sampling occasions ranged from day 1 to 10 years after transplantation.

Results: Pharmacokinetics of MPA were described by a two-compartment model with time-lagged first order absorption, and a first-order term for time-dependent CL. The model predicted mean CL to decrease from 35 L/h (CV=44%) in the first week after transplantation to 17 L/h (CV=38%) at month 6. In a covariate model without a term for time-dependent CL, a simultaneous change representative for the first 6 months after transplantation in creatinine clearance from 19 to 71 mL/min, in albumin level from 35 to 40 g/L, in haemoglobin from 9.7 to 12 g/dL and in cyclosporine pre-dose concentration from 225 to 100 ng/mL corresponded with a decrease of CL from 32 to 19 L/h. Creatinine clearance, albumin level, haemoglobin and cyclosporine pre-dose concentration explained respectively 19%, 12%, 4% and 3% within-patient variability in MPA CL.

Conclusion: By monitoring creatinine clearance, albumin level, haemoglobin and cyclosporine pre-dose concentration, structural changes in MPA exposure over time can be predicted. This knowledge can be used to optimise mycophenolate mofetil dosing.

INTRODUCTION

The pharmacokinetics of mycophenolic acid (MPA), the active immunosuppressive compound of the prodrug mycophenolate mofetil (MMF), have been extensively investigated in renal transplant recipients [1,2]. One of the important results from these studies was the elucidation of a relationship between MPA area-under-the-curve (AUC_{0-12}) and the risk of acute rejection [3-6]. Most studies also found a large between-patient variability in the pharmacokinetics of MPA [2,3,7]. This had led some clinicians to employ therapeutic drug monitoring for MMF therapy using a MPA AUC_{0-12} value above 30 mg*h/L, on which MMF dosing should be based to minimise acute rejection episodes [8]. The pharmacokinetics of MPA are further complicated by a gradual rise in MPA exposure of at least 30 to 50% from the first weeks relative to the stable period at 1 to 6 months after renal transplantation despite the use of fixed [2,7,9-12] or even reduced doses of MMF [3]. The changes are the result of decreasing MPA clearance (CL) over time [10], but the causes for this time-dependency are not completely understood. A decreasing effect of improving renal function on MPA CL has been shown [10], but also other time-varying factors may play a role. Examples are increasing albumin levels in the first months after transplantation and the gradual tapering of cyclosporine (CsA) dose and CsA target levels in that same period [7]. In order to maintain or achieve MPA target exposure despite the structural changes over time knowledge about the characteristics of the time-dependent CL is necessary. The aim of this study was to characterise the time-dependent CL of MPA and to test whether improving renal function, changes in concomitant immunosuppressive therapy and time-varying patient factors can describe the time dependency. For this purpose a population pharmacokinetic meta-analysis was conducted combining concentration-time data from the first day until after the first year posttransplant.

PATIENT AND METHODS

Patients and sampling procedures

MPA concentration-time data from six clinical studies were combined. Details about these studies have been published previously [3,4,13-16]. For all included studies approval was obtained from the respective ethics committees at the time the study was conducted. Briefly, patients in study 1 used 1, 1.5 or 1.75 g MMF twice daily in combination with CsA, which was initiated after transplantation when creatinine levels dropped below 3 mg/dL. MPA plasma samples were drawn from 18 renal transplant patients on day 1 and 20 after transplantation. Sampling times were pre-dose and at 0.5, 1, 2, 4, 8 and 12 h after MMF administration. In study 2, MPA concentration-time samples were taken pre-dose and at 0.5, 1, 2, 4, 8 and 12 h after 1 or 1.5 g MMF twice daily from 62 renal transplant recipients on days 1 and 5 after transplantation and at hospital discharge (range: day 6–day 21) [13,14]. In these patients, CsA was initiated after the first week. Study 3 was a concentration-controlled trial in which 141 CsA treated renal transplant recipients were randomised to three AUC_{0-12} target groups: low, intermediate or high [3,4]. Starting MMF doses were 0.45, 0.95 and 1.7 g MMF twice daily, where after MMF doses were adjusted aiming at AUC_{0-12} targets of respectively 16.1, 30.3 or 60.6 mg*h/L. Full concentration-time profiles were taken on day 3, 7 and 11 after transplantation. Sampling times were pre-dose and at 0.33, 0.67, 1.25, 2, 6, 8 and 12 h after oral MMF intake. On day 21 and at months 1, 2, 3, 4, and 5 after transplantation, abbreviated 2-hour profiles were drawn consisting of the first five sampling time points. In study 4, pharmacokinetic information was collected from 44 renal transplant patients after 1 g of MMF. On day 4, only a pre-dose sample was drawn followed by full concentration-time profiles taken on day 7 and at month 1, 3 and 6. Samples were drawn pre-dose and at 0.33, 0.67, 1.25, 2, 3, 4, 6, 8 and 12 h after oral MMF intake. In 14 patients, MMF was combined with CsA. In the remaining 30 patients sirolimus was used instead of CsA. In study 5, concentration-time samples were drawn at pre-dose and at 0.33, 0.67, 1.25, 2, 3, 4, 6, 8 and 12 h after 0.75 or 1 g MMF twice daily from 118 renal transplant recipients on day 7 and at month 3, 7 and 12 [15]. CsA was used as concomitant immunosuppressive therapy according to three different regimens: standard dose, low dose or low dose with withdrawal after three months. Study 6 included 85 stable renal transplant recipients treated with 1, 1.25 or 1.5 g MMF twice daily who were at least 6 months after transplantation [16]. One full concentration-time curve was taken from every patient. Sampling times were pre-dose and at 0.33, 0.67, 1.25, 2, 3, 4, 6, 8 and 12 h after MMF intake. All patients also received CsA. In every study prednisone was used according to routine practice. In study 4 and in a subset of patients in study 5, induction therapy with standard doses of daclizumab was administered. High performance liquid chromatography (HPLC) was used to measure total MPA concentrations in all drawn samples. In the six different studies, different HPLC methods were used. All six HPLC methods complied with the FDA Guidance for bioanalytical method validation [17], and had a limit of quantification for total MPA of 0.1 mg/L. The method used for quantification of total MPA in study 3 [3,4] is described in reference [18]. The intra-assay precision of this method, expressed as coefficients of variation, at the limit of quantification, 1 mg/L and 20 mg/L was 10.2%, 2.06% and 0.89%, respectively [18]. The inter-assay precision of this method at the limit of quantification, 1 mg/L and 20 mg/L was 2.85%, 1.40% and 1.85%, respectively [18].

Pharmacokinetic analysis

After appropriate pooling, all data were analysed simultaneously using the nonlinear mixed effects modelling software program NONMEM (double precision, Version V, level 1.1, GloboMax LLC, Hanover, USA). Because NONMEM estimated pharmacokinetic parameters for MPA, MMF doses were converted to the equivalent MPA content by multiplying MMF dose by 0.739. Data were logarithmically

transformed (natural logarithm) and the first order (FO) estimation method was used throughout the entire model building process, because of the high computational intensity of the first order conditional estimate method (FOCE).

Basic model

The first step of the analysis was the development of a compartmental model, in which also pharmacokinetic variability and time dependent CL of MPA were quantified. Pharmacokinetic parameters were estimated in terms of CL, central and peripheral volumes of distribution (V1 and V2, V3) and intercompartmental clearances (Q2, Q3). Since bioavailability (F) could not be quantified, apparent oral values of CL, Q and V were estimated, these values are the ratios CL/F, Q/F and V/F respectively. Between-patient variability in these parameters was estimated using exponential models. Covariance was estimated between the parameters for between-patient variability. Also within-patient variability of the pharmacokinetic parameters was modelled [19]. The difference between observed and model predicted MPA concentrations (residual variability) was modelled as additive to the log of the observed MPA concentration [20].

The time-dependency of MPA CL was modelled as a first order process shown in equation 1:

$$CL\ (L/h) = \theta_{ss} + \theta_{\Delta} * \exp(-\theta_{rate} * \text{time (h)}) \quad \text{(Eq. 1)}$$

In which θ_{ss} is the population value of MPA CL when steady-state of CL has been reached, θ_{Δ} is the change of MPA CL from its steady-state value and θ_{rate} is a first order rate constant determining the rate with which CL changes over time.

Covariate model

The second step was an analysis of the influence of covariates on the pharmacokinetic parameters. Relationships were investigated in the basic model without time-dependent CL and according to a two-stage approach: in the first stage covariates were introduced separately and tested for their significance in an univariate analysis. Continuous covariates, which could take the value 0, for example CsA dose, were modelled in a proportional manner with the covariate value centered around its population median value as shown in equation 2:

$$CL_{ij}\ (L/h) = \theta_{pop} * (1 + \theta_{CsA\ dose} * (\text{CsA dose} - 325)) \quad \text{(Eq. 2)}$$

where θ_{pop} is the MPA CL in individuals with the median CsA daily dose of the population (325 mg) and $\theta_{CsA\ dose}$ is the fractional change of CL per mg CsA.

Continuous covariates that could not take the value 0, for instant weight, were modelled in an exponential manner, which allows for estimation of nonlinear relationships (equation 3):

$$CL_{ij}\ (L/h) = \theta_{pop} * (\text{Weight}/71)^{\theta_{Weight}} \quad \text{(Eq. 3)}$$

in which θ_{pop} is the MPA CL in individuals with the median weight of the population (71 kg) and θ_{Weight} is an exponent determining the shape of the relationship. Categorical variables, like for example gender, were modelled as shown in equation 4:

$$CL_{ij}\ (L/h) = \theta_{pop} * \theta^{gender} \quad \text{(Eq. 4)}$$

where θ_{pop} is the population value for MPA CL in females (gender = 0) and θ^{gender} is the fractional change of CL in males (gender = 1). Covariates with less than 4% of missing data were imputed with population median values of the following time frames after transplantation: day 0 to 4, day 5 to 8, week 2, week 3, week 4, month 2, month 3, month 4, month 5, month 6, the second half of the first year and beyond the first year. The population median used for imputation corresponded with the moment after transplantation on which covariate data were missing. The value of 4% of missing data was arbitrarily chosen. When more than 4% of data were missing, an adjustment was made in the model according to equation 5, which is derived from equation 3:

$$CL_{ij}(L/h) = \theta_{pop} * ((Weight/71)^{\theta_{Weight} * FLAG_{ij}}) * \theta^{1-FLAG_{ij}} \quad \text{(Eq. 5)}$$

where the indicator variable FLAG*ij* has the value of 1 when covariate data are present for the i^{th} individual on the j^{th} occasion and 0 when data are missing. The result is that observed MPA concentration-time data from patients with missing covariate data on a certain occasion are ignored for estimation of the effect of the covariate (θ_{Weight}), and are used in the estimation of $\theta^{1-FLAG_{ij}}$. $\theta^{1-FLAG_{ij}}$ has no further pharmacokinetic meaning.

Whether a covariate had a significant effect was determined by the difference between the minimum value of objective function (OFV) generated by NONMEM for two hierarchical models [21]. When inclusion of a covariate caused a decrease of the OFV of >10.8 units ($p<0.001$, 1 degree of freedom), the covariate was considered to be statistically significant. In addition to this, a reduction in the estimate for between- and within-patient variability was a criterion for covariate selection.

In the second stage, all covariates selected during the first stage were included in an intermediate model. Covariates were excluded separately from the intermediate model in a backward elimination procedure. If the elimination of a covariate significantly worsened the fit of the model (increase of OFV>10.8), the covariate remained in the model. The result of the backward elimination procedure was the final model.

The final model was refined by testing models for covariates structurally changing over time as described by Wählby et al [22] when visual inspection of a correlation between time and covariates indicated such a trend. Models for time-varying covariates may provide more valuable information than time-constant covariate models (equation 2 and 3), because the effect of a covariate on between-patient variability in a pharmacokinetic parameter can be estimated separately from the effect of that covariate on within-patient variability [22]. They also allow the magnitude of an effect of a covariate on a pharmacokinetic parameter to vary between individuals, thus estimating between-patient variability as shown in equation 6, which is derived from equation 3 [22]:

$$CL_{ij}(L/h) = \theta_{pop} * (Weight/71)^{\theta_{Weight} * \exp(\eta_{Weight})} \quad \text{(Eq. 6)}$$

where η_{Weight} is a random variable with mean 0 and variance ω^2 allowing the effect of weight on CL (θ_{Weight}) to vary between individuals.

Finally, the time-dependent CL term (equation 1) was included in the final model again to test the hypothesis that the selected covariates could describe all time-dependency in CL.

The adequacy of the developed NONMEM models was evaluated using the precision of the parameter estimates and goodness-of-fit plots [23]. Goodness-of-fit plots were generated in Xpose (version 3.010), an S-PLUS based (Version 6.2, professional edition, Seattle, USA) modelling aid [24].

Model validation

The stability and the performance of the final model were checked with an internal validation of the final model, using the bootstrap resample technique [25]. During a bootstrap procedure approximately 65% of the original data are resampled with replacement, which produces different combinations of data sets. The final model is fitted to each artificial sample and estimates for all parameters are produced. For this purpose the bootstrap option in the software package Wings for NONMEM (Nick Holford, version 405, November 2003, Auckland, New Zealand) was used.

RESULTS

Basic model

In total, 1894 concentration-time profiles were available from 468 renal transplant recipients, ranging from 1 to 9 profiles per patient. Patient demographics are summarised in table 1. Sampling occasions varied from day 1 to day 3795 (>10 years) after transplantation and MMF doses ranged from 250 mg to 2200 mg twice daily. Data were simultaneously fitted to several compartmental models. A two-compartment model with a time lagged first order absorption resulted in the best goodness-of-fit. Typical population estimates for the pharmacokinetic parameters for this basic model are summarised in table 2. A coefficient of variation for between- and within-patient variability could be estimated for the first order absorption rate constant (Ka), V1, CL and Q (table 2). Of note, estimates for within-patient variability (κ) in this model showed a skewed distribution with mean (±standard error) values significantly >0 on early occasions after transplantation (e.g. 0.074±0.010 on the first occasion, $p<0.001$) and mean values significantly <0 on later occasions (e.g. –0.037±0.010 for the last occasion, $p<0.001$).

When time-dependent CL (equation 1) was introduced in this basic model, the goodness-of-fit of the model improved and estimates for between- and within-patient variability decreased (table 2). The drop of OFV was 291 units, however formally this cannot be applied as a criterion for goodness-of-fit in this situation. Within-patient variability for CL decreased from 34 to 24% and κ estimates had mean (±standard error) values much closer to 0 on all occasions relative to the basic model without the time-dependent CL, e.g. -0.01±0.01 on the first occasion. The time-dependent CL was characterised as follows (equation 7):

$$CL\ (L/h) = 20 + 14 * \exp(-8.8*10^{-4} * \text{time (h)}) \qquad \text{(Eq. 7)}$$

The value for θ_{rate} of $8.8*10^{-4}$ h^{-1} can be derived with: $\ln 2 / 8.8*10^{-4} = 788$ h = 33 days, thus providing an estimate for the half-life of MPA CL. According to equation 7, the model predicts a MPA CL of 34 L/h ($\theta_{ss} + \theta_{\Delta}$) immediately after renal transplantation, which decreases over a period of 165 days (5 times the MPA CL half-life) to 20 L/h (table 2, figure 1a).

Table 1 Patient demographics and biochemical parameters on four sampling occasions after renal transplantation (results presented as median (range))

Characteristics	Missing data	Day 0-4	Month 1	Month 6	Year 1
Gender (n)					
Male	0%	157	119	87	104
Female	0%	89	69	47	67
Race (n)					
Caucasian	0%	217	168	121	131
Black	0%	17	7	4	37
Other	0%	12	13	9	3
Diabetes mellitus (n)	0%	49	17	15	16
DGF (n)	3.8%	34	-	-	-
Use of antacids (n)	0%	56	24	0	1
Use of proton pump inhibitors (n)	0%	7	13	0	0
Use of H_2-antagonists (n)	0%	66	82	3	5
Use of antiviral agents (n)	0%	68	8	1	0
Use of sirolimus (n)	20%	21	27	17	2
Age (years)	0%	50 (18-72)	50 (19-70)	49 (28-70)	52 (22-73)
Body weight (kg)	0.1%	71 (37-151)	68 (38-151)	80 (42-151)	75 (49-122)
Serum creatinine (µmol/L)	2.9%	424 (66-1379)	128 (53-913)	124 (62-195)	125 (52-221)
Creatinine clearance (mL/min)	2.9%	19 (4-132)	55 (7-203)	71 (44-132)	64 (34-113)
Plasma albumin (g/L)	14%	35 (23-51)	35 (26-50)	36 (29-45)	42 (31-48)
Serum ALT (U/L)	12%	17 (2-653)	17 (4-144)	25 (10-128)	20 (11-1759)
Serum total bilirubin (mg/dL)	12%	0.5 (0.2-3.0)	0.6 (0.1-1.9)	0.5 (0.1-1.6)	0.7 (0.2-3.3)
Serum alkaline phosphatase (U/L)	12%	64 (17-870)	86 (25-221)	99 (46-218)	171 (41-347)
Red blood cell count ($x10^{12}$/L)	20%	3.2 (1.5-4.8)	3.4 (2.1-4.9)	4.3 (3.5-5.9)	4.4 (3.7-9.5)
Haemoglobin (g/dL)	3.5%	9.7 (4.9-17)	11 (6.7-15)	12 (9.6-18)	13 (7.8-18)
Prednisone daily dose (mg)	11%	30 (20-1365)	19 (7.5-35)	10 (0-10)	9.4 (0-10)
Cyclosporine daily dose (mg)	4.8%	530 (0-1000)	350 (0-1400)	100 (0-200)	138 (0-300)
Cyclosporine pre-dose concentration (ng/mL)	5.3%	171 (0-806)	237 (0-571)	93 (0-316)	155 (0-1337)
Patients not using cyclosporine (n)	4.8%	102	27	17	34
mycophenolate mofetil dose (mg twice daily)	0%	1150 (400-2200)	1000 (250-2200)	1000 (1000-1000)	1000 (250-1250)

Data were collected from 468 renal transplant recipients participating in 6 clinical studies. Because of different moments of pharmacokinetic assessment after transplantation in the studies, the number of individuals from whom data were available differs for the four presented occasions. n = number of renal transplant recipients, DGF = delayed graft function. Normal values as used at Erasmus MC, Rotterdam, The Netherlands for creatinine: 65-115 µmol/L for males and 55-90 µmol/L for females, for plasma albumin: 35-50 g/L, for serum ALT: <41 U/L for males and <31 U/L for females, for serum total bilirubin: <1 mg/dL, for serum alkaline phosphatase: <120 U/L, for red blood cell count: 4.4-5.6$x10^{12}$/L for males and 3.9-4.9$x10^{12}$/L for females, for haemoglobin: 13.8-16.9 g/dL for males and 12.1-15.3 mg/dL for females.

Table 2 Parameter estimates for the basic model with and without time-dependent CL, the final model and the bootstrap procedure with their coefficients of variation (CV)

Parameter	Basic model without tdCL	Basic model with tdCL	Final model	500 Bootstrap replicates of the final model
	Estimate (CV%)			Mean estimate (CV%)
Minimum OFV	454.9	163.5	-505.5	
PK parameters				
Ka (h^{-1})	4.4 (6%)	3.6 (5%)	4.0 (7%)	4.0 (8%)
V1 (L)	131 (8%)	111 (10%)	69 (6%)	70 (4%)
CL (L/h)	27 (4%)	-	23 (2%)	23 (3%)
V2 (L)	449 (10%)	388 (9%)	298 (8%)	299 (8%)
Q (L/h)	64 (11%)	49 (10%)	34 (7%)	35 (9%)
T_{lag} (h)	0.21 (2%)	0.24 (1%)	0.24 (1%)	0.23 (5%)
Time-dependent parameters				
CL_{ss} (L/h)	-	20 (3%)	-	-
CL_{Δ} (L/h)	-	14 (6%)	-	-
θ_{rate} (h^{-1})	-	$8.8*10^{-4}$ (12%)	-	-
Between-patient variability				
Ka (%)	156 (13%)	112 (15%)	101 (14%)	109 (12%)
V1 (%)	148 (13%)	113 (17%)	90 (16%)	94 (9%)
CL (%)	44 (9%)	38 (9%)	36 (9%)	36 (5%)
Q (%)	104 (25%)	78 (22%)	60 (21%)	61 (12%)
Covariance (r) in estimates for between-patient variability				
r_{Ka-V1}	0.74 (15%)	-	0.45 (28%)	0.49 (18%)
r_{Ka-CL}	0.14 (67%)	-	-0.13 (67%)	-0.13 (66%)
r_{Ka-Q}	0.54 (29%)	-	0.21 (72%)	0.23 (66%)
r_{V1-CL}	0.40 (21%)	0.44 (19%)	0.45 (17%)	0.43 (16%)
r_{V1-Q}	0.46 (27%)	0.55 (24%)	0.33 (30%)	0.31 (34%)
r_{CL-Q}	0.50 (19%)	0.56 (17%)	0.54 (18%)	0.51 (19%)
Within-patient variability				
Ka (%)	137 (12%)	124 (14%)	116 (10%)	117 (6%)
V1 (%)	89 (11%)	80 (13%)	71 (12%)	71 (6%)
CL (%)	34 (7%)	24 (10%)	21 (10%)	21 (5%)
Q (%)	77 (23%)	74 (22%)	41 (39%)	41 (21%)
Residual variability				
Additive error (mg/L)*	0.42 (2%)	0.44 (2%)	0.44 (2%)	0.43 (2%)
Covariate effects				
CsA dose on Ka	-	-	$9.8*10^{-4}$ (20%)	$9.4*10^{-4}$ (20%)
Factor for missing data			0.99 (12%)	0.98 (14%)
Albm on V1	-	-	-1.2 (17%)	-1.2 (17%)
Factor for missing data	-	-	1.0 (6%)	1.1 (6%)
CrCl on V1	-	-	-0.49 (10%)	-0.48 (10%)
Antacids on V1	-	-	1.4 (8%)	1.4 (8%)
CsA pre-dose concentration on CL	-	-	$4.8*10^{-4}$ (16%)	$4.8*10^{-4}$ (16%)
Factor for missing data	-	-	0.96 (5%)	0.96 (5%)
Albm on CL	-	-	-0.72 (13%)	-0.71 (13%)
Factor for missing data	-	-	1.1 (3%)	1.1 (3%)
CrCl on CL	-	-	-0.22 (7%)	-0.22 (8%)
Hb on CL	-	-	-0.48 (16%)	-0.48 (16%)
Between-patient variability in covariate effects				
Albm on CL (%)	-	-	66 (29%)	67 (15%)
CrCl on CL (%)	-	-	112 (44%)	115 (23%)

*Residual variability is on a natural logarithmic-scale as data were logarithmically transformed. tdCL = time-dependent CL, OFV = objective function, Ka = first order absorption rate constant, V1 = central distribution volume, CL = clearance,

V2 = peripheral distribution volume, Q = intercompartment clearance, T_{lag} = lag time, CL_{ss} = steady-state value of CL, CL = change of MPA CL from its steady-state value, θ_{rate} = first order rate constant determining the rate with which CL changes over time, *r* = correlation coefficient, CrCl = creatinine clearance, Albm = plasma albumin concentration, Hb = haemoglobin, CsA = cyclosporine. Typical Ka, V1 and CL values can be calculated as follows:

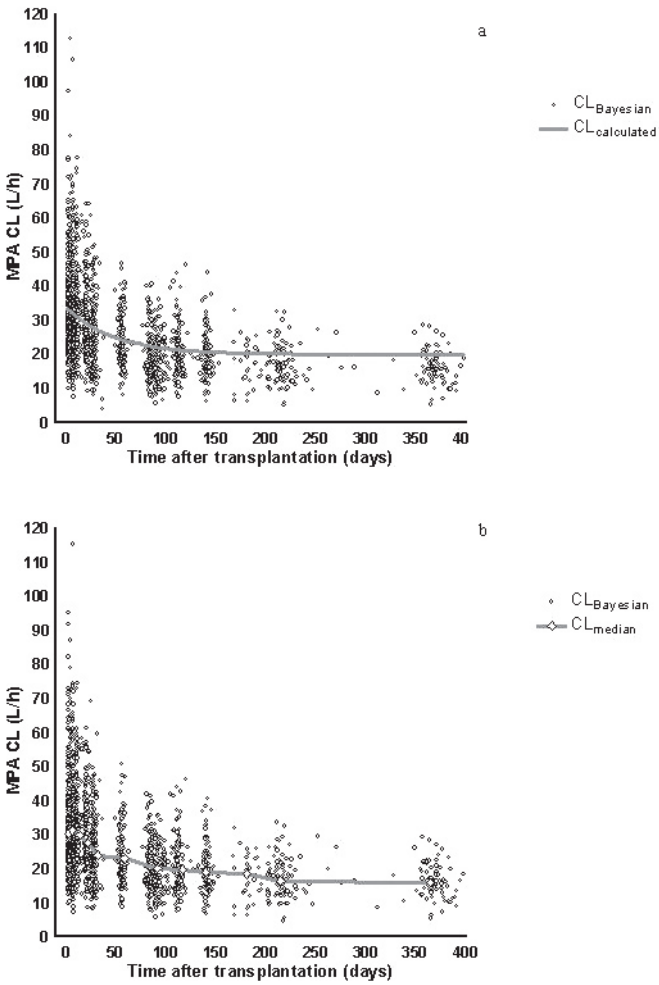
$$Ka = 4.0 * (1 + 9.8*10^{-4} * (CsA\ dose-325) * FLAG3ij) * 0.99^{1-FLAG3ij}$$

$$V1 = 69 * (CrCl/50)^{-0.49} * (Albm/38)^{-1.18 * FLAG1ij} * 1.0^{1-FLAG1ij} * 1.4^{antacids}$$

$$CL = 23 * (CrCl/50)^{-0.22 * \exp(\eta_{CrCl})} * (Albm/38)^{-0.72 * \exp(\eta_{Albm}) * FLAG1ij} * 1.1^{1-FLAG1ij} * (1 + 4.8*10^{-4} * (CsA\ pre-dose-200) * FLAG2ij) * 0.96^{1-FLAG2ij} * (Hb/11)^{-0.48}$$

where FLAG1*ij*, FLAG2*ij* and FLAG3*ij* are 1 when Albm, CsA pre-dose concentration and respectively CsA dose data are present and 0 when covariate data are missing.

Figure 1 a: Time after renal transplantation versus mycophenolic acid (MPA) clearance (CL) based on the basic model with time-dependent CL. Open circles are individual Bayesian estimated CL-values and the solid line represents the model estimated relationship between time and CL according to equation 7. b: Time after renal transplantation versus MPA CL based on the final model. Open circles are Bayesian estimated CL-values and the solid line represents the median course over time of MPA CL.



Covariate model

Although equation 7 can describe the changes in MPA CL over time, a model in which covariates describe the time-dependency in CL is more useful from a perspective of therapeutic drug monitoring. In order to identify covariates which could describe the time-dependent CL, the following variables were tested in the basic model without time-dependent CL: patient race (Caucasian, African-American, other), age, gender, weight, plasma albumin level (Albm), ALT, bilirubin, alkaline phosphatase, haemoglobin (Hb), red blood cell count, delayed graft function (DGF, defined as the need for dialysis during the first two weeks after transplantation), diabetes mellitus, MMF dose, CsA dose, CsA pre-dose concentration, corticosteroid dose, the use of antiviral agents, antacids, H_2-antagonists and sirolimus. Renal function was tested by 1) calculation of the creatinine clearance (CrCl) according to Cockcroft and Gault [26] and 2) calculation of the glomerular filtration rate through the abbreviated Modification of Diet and Renal Disease method (aMDRD) [27]. Because univariate correlations between pharmacokinetic parameters and CrCl explained more variability than correlations with aMDRD, CrCl was used as measure for renal function and aMDRD was rejected.

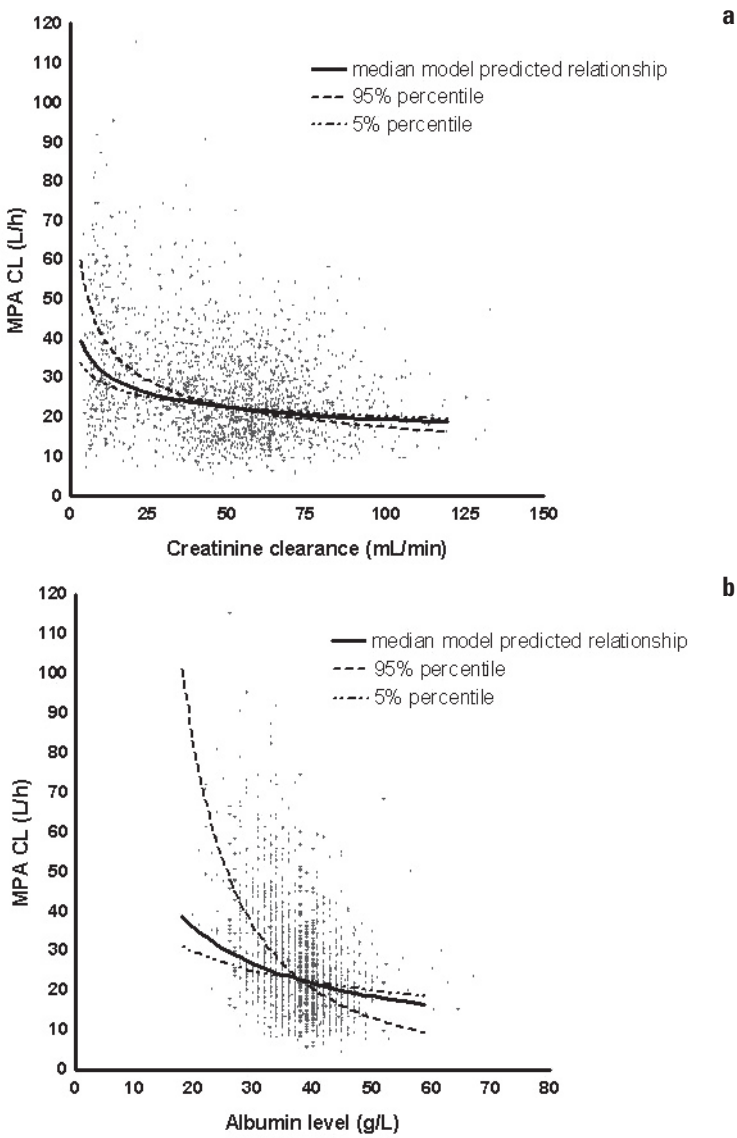
After the backward elimination procedure, CsA dose was significantly correlated with Ka; CrCl, Albm and the use of antacids were significantly correlated with V1; CrCl, Albm, CsA dose and Hb had a significant effect on CL (table 2). Exclusion of each of these covariates during the backward elimination procedure resulted in an increase of OFV of at least 20 units ($p<0.001$). Despite a weaker correlation of CsA pre-dose concentration with CL compared to CsA dose in the backward elimination procedure, the former was included in the covariate model as exposure to CsA, and thereby adequacy of CsA therapy in an individual patient, is better reflected by the pre-dose concentration than by CsA dose. Equation 8 shows the results of the covariate analysis for MPA CL:

$$CL = 23 * (CrCl/50)^{-0.22 * \exp(\eta_{CrCl})} * (Albm/38)^{-0.72 * \exp(\eta_{Albm})} * FLAG1_{ij} * 1.1^{1-FLAG1_{ij}} * (1 + 4.8*10^{-4} * (CsA\ pre\text{-}dose-200) * FLAG2_{ij}) * 0.96^{1-FLAG2_{ij}} * (Hb/11)^{-0.48} \quad \text{(Eq. 8)}$$

where FLAG1*ij* is 1 when Albm covariate data are present and 0 when covariate data are missing. The same applies for FLAG2*ij* with regard to CsA pre-dose concentration data. In equation 8, a change of CrCl from 19 to 71 mL/min in a typical patient (Albm: 38 g/L, Hb: 11 mg/dL, CsA pre-dose: 200 ng/mL) correlated with a decrease in MPA CL from 28 to 21 L/h (figure 2a). This relationship explained no between-patient variability, but 19% within-patient variability, as determined by comparison of the variability estimates in the final model with and without the relationship. An increase of Albm from 35 to 40 g/L was associated with a decrease of CL from 24 to 22 L/h (figure 2b). This correlation explained 5% between and 12% within-patient variability. An increase of Hb from 9.7 to 12 mg/dL correlated with a decrease in CL from 24 to 22 L/h. Another 5% between and 4% within-patient variability was explained by this correlation. Finally, a decrease of CsA pre-dose concentration from 225 to 100 ng/mL was associated with a decrease of MPA CL from 23 to 22 L/h. This relationship explained 3% between and 3% within-patient variability. Importantly, the mentioned changes in covariate values are representative for the changes within a patient during the first six months after transplantation (table 1). When these changes occur simultaneously, equation 8 estimated a decrease of MPA CL from 32 to 19 L/h, indicating that the covariates can describe the time-dependent decrease of CL. This is also shown in figure 1b.

During model refinement, introduction of between-patient variability in the effect of CrCl and Albm on MPA CL according to equation 6 resulted in a significant improvement of the fit: OFV decreased with 28 and 36 units respectively ($p<0.001$) and the estimate for within-patient variability in CL decreased from 23% to 20% [21]. Between-patient variability of the effect of CrCl and Albm on CL was 66% and 112%, respectively (equation 8, table 2, figure 2a and b).

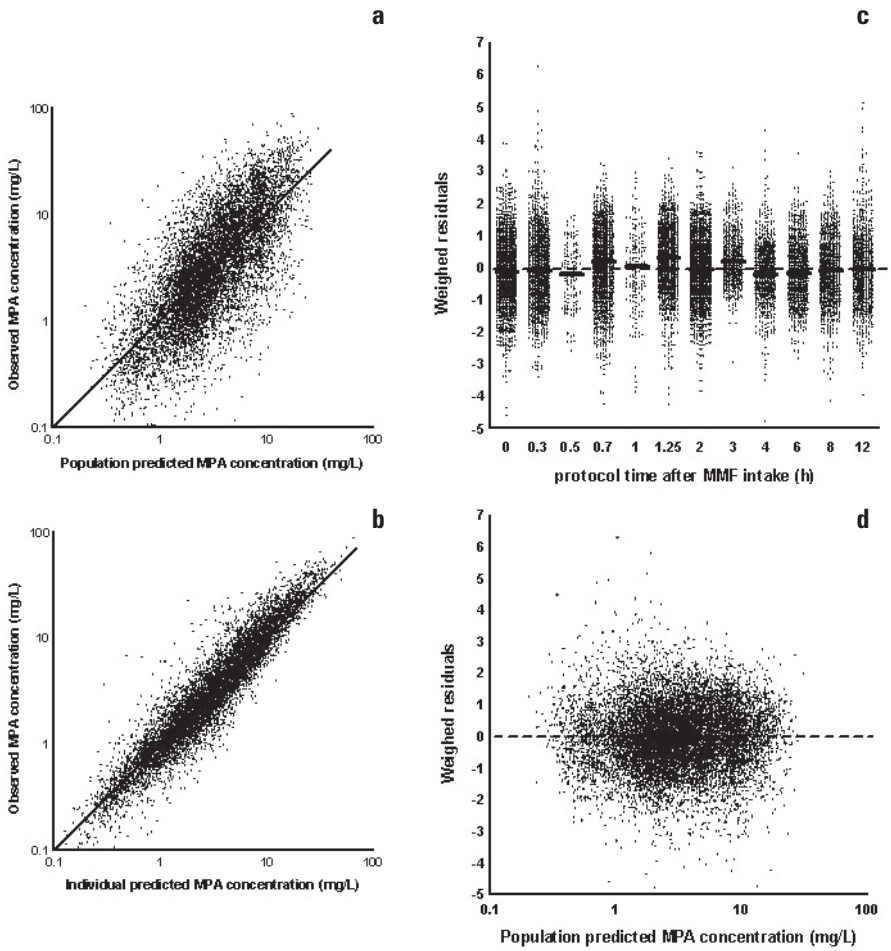
Figure 2 a: correlation between creatinine clearance (CrCl) and mycophenolic acid (MPA) clearance (CL) as identified in the final model. The solid line represents the model predicted relationship for a typical patient with an albumin level of 38 g/L, a haemoglobin of 11 g/dL and a cyclosporine pre-dose concentration of 200 ng/mL according to equation 8. b: correlation between albumin level and MPA CL as identified in the final model. The solid line represents the model predicted relationship for a typical patient with a CrCl of 50 mL/min, a haemoglobin of 11 g/dL and a cyclosporine pre-dose concentration of 200 ng/mL according to equation 8. The broken lines in both a and b represent the 5 and 95 percentiles of the between-patient variability in both relationships, illustrating that the magnitude of the effect of CrCl and albumin level on MPA CL varies between individuals. This means that the same change in CrCl or albumin level can have a large effect on MPA CL in one patient (95 percentile, – – – – – – –), while hardly any effect can be present in another patient (5 percentile, – · – · – · – · –). The between-patient variability in the effects of CrCl and albumin level on MPA CL was modeled according equation 6. Because CrCl values above 120 mL/min are not physiologically plausible in renal transplant patients, the solid and broken lines in a are cut at 120 mL/min. Data points with a CrCl above 150 mL/min are not shown (n=4).



Introduction of the time-dependent CL term (equation 1) into the final model produced an OFV which was 160 units lower than the same model without the time-dependent term. Although this suggests an improvement of the fit, this model did not converge successfully.

The covariate model without the time-dependent CL term did converge successfully, and was capable of estimating the time-dependent CL through the covariate effects as shown in figure 1b and had κ estimates with mean (±standard error) values close to 0 on all occasions (e.g. -0.003±0.005 on the first and -0.006±0.003 on the last occasion). For these reasons, this model was accepted as the definitive final model.

Figure 3 Goodness-of-fit plots for the final model. a: population predicted mycophenolic acid (MPA) concentration versus observed MPA concentration; b: individual Bayesian predicted MPA concentration versus observed MPA concentration; c: protocol sample time versus weighed residuals; d: population predicted MPA concentration (PRED) versus weighed residuals. The solid lines in a and b represent the line of identity. The dotted lines in c and d represents the line y = 0. The small solid horizontal lines in c represent the median weighed residual for the protocol sampling times.



The goodness-of-fit plots of the final model are presented in figures 3a to d and show a random distribution of data points around the line of identity (figures 3a and b) and the line WRES=0 (figures 3c and d), indicating that the model is free from bias. However, figure 3c shows that maximum MPA concentrations, occurring between 0.7 and 1.25 h after MMF administration, are slightly underestimated by the model. Because this minor amount of model misspecification is within acceptable limits (95.2% of all WRES fall between –2 and 2), the model is believed to describe the data adequately.

Model validation

500 bootstrap replicates were done and all converged successfully. The results showed very similar parameter estimates compared to the population estimates of the final model (table 2). No bootstrapped parameter estimate differed more than 9% from its corresponding estimate in the final model.

DISCUSSION

The pharmacokinetics of MPA are complicated as a result of large between-patient variability in MPA exposure and a gradual rise of exposure over time after renal transplantation despite a fixed MMF dose [2,3,8,10]. The time-dependent changes of MPA pharmacokinetics cause at least a 30% to 50% increase of MPA $AUC_{0\text{-}12}$ during the first weeks after transplantation [2]. This study showed that the time-dependent change of exposure to MPA is caused by decreasing MPA CL, which can be described by a combination of improving CrCl, increasing Albm, increasing Hb and decreasing CsA pre-dose concentrations during the first half year after transplantation. A number of observations from the present analysis provide evidence for this statement. First, while the final model successfully minimised without error messages, a model in which both the time-dependent CL term and the covariate effects were included, did not converge successfully. The latter could not be resolved by changing the significant digits or the initial estimates of the model. Unsuccessful convergence could be indicative for over-parameterisation suggesting that the data do not contain sufficient information to support reliable estimation of all the parameters in the model. Second, the estimated change of MPA CL as predicted by the basic model with the time-dependent CL-term (equation 7) from 34 to 20 L/h over a period of the first 165 days after transplantation, is very similar to the change in CL from 32 L/h to 19 L/h in the final model described by typical changes in covariate values over that same period of time (table 1, figures 1a and b). Third, while estimates for within-patient variability (κ) should have a normal distribution around 0, estimates of κ in the basic model without time-dependent CL showed a skewed distribution with mean (±standard error) values >0 on early occasions after transplantation and mean values <0 on later occasions. This suggests that the structural time-dependent effect is taken into account by the estimates for within-patient variability. In contrast, κ estimates in the basic model with time-dependent CL as well as in the final model had mean values much closer to 0 on all occasions. This indicates that equations 7 and 8 account for the time dependent effect now instead of κ estimates.

The influence of CrCl on MPA CL may be explained through an effect on MPA protein binding. Acidosis and uraemia are associated with impaired renal function and will decrease MPA binding to albumin [10]. Moreover, accumulation of the glucuronide metabolite of MPA (MPAG) during renal impairment will displace MPA from its albumin binding sites [28]. As MPA is supposed to be a restrictively cleared drug, the resulting increase of MPA free fraction leads to an increase of the amount of MPA available for glucuronidation and hence to a higher MPA CL [10,29,30]. Also the increase in MPA CL following low albumin levels is likely to be due to an increased free fraction. The same mechanism might explain the identified effect of Hb on CL, but the fraction MPA found in red blood cells was very small in an *in vitro* study (0.001%), making it uncertain how Hb affects MPA CL [28].

The effect of CsA pre-dose concentration on MPA CL can be explained by the inhibitory effect of CsA on the enterohepatic recirculation (EHC) of MPA [31-33].

The use of antacids was associated with a 40% higher V1 (table 2). Antacids containing aluminium or magnesium ions are known to be capable of interacting with co-administered drugs through formation of non-absorbable complexes. As V1 represents apparent oral V1 (V1/F), patients who used antacids may have a 40% lower F as a consequence of binding of MPA by these drugs in the gut [1]. Although this is a likely explanation, an effect of antacids on apparent oral CL, which would also be expected then, could not be proven. This makes the mechanism of the effect of antacids on V1 uncertain.

The covariates correlated with MPA CL identified in the present analysis, CrCl, Albm, Hb and CsA pre-dose concentration, are frequently measured in every renal transplant recipient to monitor the clinical status of patients. The easy availability of these parameters offers opportunities for clinicians to monitor and optimise MMF therapy. First, immediately after renal transplantation (first week) the population CL is 32 L/h, resulting in an AUC_{0-12} of 31 mg*h/L after a standard MMF dose of 1000 mg. Consequently, a large part of the population will be below the AUC_{0-12} target limit of 30 mg*h/L [8], most likely those patients with poor renal function, low Albm, low Hb and those concurrently treated with CsA. It is therefore advisable to measure MPA exposure in these patients to select the ones who need a dose increase to reach target exposure. Second, by monitoring CrCl, Albm, Hb and CsA pre-dose concentrations, the clinician can get a feeling about changes in MPA CL within a patient over time, which will mostly be a gradual decrease during the first 5 to 6 months after transplantation. Measurement of MPA exposure after the mentioned variables have stabilised in a patient, or after CsA dose tapering can identify those with high AUC_{0-12} levels (above 60 mg*h/L [8]) and who may benefit from a MMF dose reduction. Importantly, as the recommended target AUC_{0-12} levels for MPA are only validated for the first half year after transplantation, clinical studies relating MPA exposure to long-term outcomes would be of benefit [8].

Despite having identified significant correlations between MPA CL and CrCl, Albm, Hb and CsA pre-dose concentration, therapeutic drug monitoring of MPA remains advisable to base MMF dose on for the following reasons. 1) As shown in figure 2a and b, large between-patient variability was estimated in the effect that CrCl and Albm had on MPA CL (66 and 112%, respectively). This means that the same change in CrCl or Albm does not necessarily lead to the same change in CL in all patients [22]. Consequently, a change in these covariates is not an indication for dose adjustment, but merely provides an indication to measure MPA concentration to check whether exposure has changed and whether MMF dose adjustments are needed to maintain target exposure [8]. 2) The identified covariates explained too little between- and within-patient variability in MPA CL (range: 0-5% explained between-patient variability and 3-19% explained within-patient variability) to serve as a basis for MMF dose selection.

The validity of the final model was confirmed by the results of the bootstrap procedure, and the parameter estimates and covariate effects are, in general, in agreement with previous population pharmacokinetic studies of MPA [33-39]. However, figures 3a to d show an underestimation of the observed maximum MPA concentrations (C_{max}). The likely reason is that with orally administered drugs conventional compartmental models with a lag time and a first or zero order absorption rate constant are often not able to accurately predict a rapid initial increase in plasma concentration [40]. Models with time-dependent absorption or with a dual sequential first order absorption process may offer a solution, but when they were tested on a subset of the data, the fit was not significantly improved.

Only two of the previous population pharmacokinetic studies of MPA included EHC in their final model [33,37], which is a well-known feature of MPA pharmacokinetics [1]. The present final model did not account for an EHC. Nevertheless, ignoring the EHC of MPA did not result in gross model

misspecification, as shown in figure 3a to d. A reason may be that 438 out of the 468 patients were treated with CsA during at least a part of the course of the study, and CsA is known to inhibit the EHC of MPA [31,32]. However, a small overestimation of low MPA concentrations occurring at the end of a dosing interval, visible in figure 3a to d, may indeed be the result of the inability to fit secondary peak concentrations.

CONCLUSION

In conclusion, on the basis of a population pharmacokinetic meta-analysis, time-dependent CL of MPA was characterised. The time-dependent CL could be described by changes in CrCl, Albm, Hb and CsA pre-dose concentration. By monitoring these variables the clinician can get a feeling about the changes of exposure to MPA over time. This offers rational tools which help the clinician decide when to measure MPA exposure in a certain patient, to reach and maintain the pre-defined MPA target concentrations with a limited number of sampling occasions [41,42].

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Chapter 2.5

Cyclosporine interacts with mycophenolic acid by inhibiting the multidrug resistance-associated protein 2

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ABSTRACT

In mycophenolate mofetil (MMF)-treated organ transplant recipients, lower mycophenolic acid (MPA) plasma concentrations have been found in cyclosporine (CsA) compared with tacrolimus (Tac)-based immunosuppressive regimens. We previously demonstrated that CsA decreases exposure to MPA and increases exposure to its metabolite MPA-glucuronide (MPAG), possibly by interfering with the biliary excretion of MPAG. To elucidate the role of the multidrug resistance-associated protein (Mrp)-2 in the interaction between MMF and CsA, we treated three groups of ten Mrp2-deficient rats (TR- rat) for 6 days with either vehicle, CsA (8 mg/kg) or Tac (4 mg/kg) by oral gavage. Hereafter, co-administration with MMF (20 mg/kg) was started in all groups and continued through day 14. The 24h MPA/MPAG area-under the concentration-time curve (AUC) was determined after single (day 7) and multiple MMF doses (day 14). On both study days, there were no significant differences in the mean MPA and MPAG AUC between CsA and Tac-treated animals. We conclude that the pharmacokinetics of MMF are comparable in Mrp2-deficient rats receiving either CsA or Tac as co-medication. This finding suggests that CsA-mediated inhibition of the biliary excretion of MPAG by the Mrp2 transporter is the mechanism responsible for the interaction between CsA and MMF.

INTRODUCTION

Mycophenolate mofetil (MMF) is a pro-drug that is rapidly and almost completely absorbed from the gut where it is de-esterfied to form mycophenolic acid (MPA), the active immunosuppressant. MPA is converted by the uridine diphosphate glucuronosyl transferase (UDPGT) enzyme family into 7-hydroxy-glucuronide mycophenolic acid (MPAG) which is excreted into bile and is not pharmacologically active. In the gut, bacterial deconjugation transforms MPAG back into MPA, which is absorbed from the colon. Because of this enterohepatic circulation, the initial MPA plasma concentration peak at 1 hour is followed by a second increase in the MPA plasma concentration, occurring 6 to 12 hours after oral administration. In human subjects, interference with the enterohepatic circulation reduces the MPA area-under the concentration *vs.* time-curve (AUC) by 35-40% [1,2]. Finally, the majority of the absorbed MMF is eliminated by the kidneys as MPAG [1,2].

We and others previously demonstrated that co-administration of MMF with CsA to solid organ transplant recipients leads to a reduction of MPA plasma concentrations and an increase in the plasma levels of MPAG as compared with patients treated with MMF plus Tac or corticosteroids [3-6]. These clinical findings were confirmed in an animal study that compared MPA and MPAG exposure between Lewis rats that were treated with MMF plus CsA, MMF plus Tac or MMF plus placebo [7]. Rats in the MMF plus Tac and MMF plus placebo groups showed a second peak in the plasma MPA AUC, consistent with enterohepatic recirculation. In contrast, animals treated with MMF plus CsA showed a marked reduction of the second MPA peak, resulting in a significantly lower mean MPA AUC. Furthermore, co-administration of CsA significantly increased the AUC of MPAG, suggesting a CsA-induced inhibition of MPAG excretion into bile [7].

At present, the exact mechanism responsible for the pharmacokinetic interaction between MMF and CsA is unknown. We hypothesised that CsA impairs biliary MPAG elimination through inhibition of the multidrug resistance-associated protein (MRP) 2 (or ABCC2, previously known as canalicular multispecific organic anion transporter). MRP2 is expressed at the apical (canalicular) surface of hepatocytes, where it functions to excrete endogenous conjugates as well as conjugation products of drug metabolism into bile [8,9]. Evidence for the implication of MRP2 in the MMF-CsA interaction comes from the observation that CsA can cause a conjugated hyperbilirubinemia (a MRP2 substrate) *in vivo* and is an inhibitor of

MRP2 function *in vitro* [10-12]. Furthermore, it was recently demonstrated that Eisai hyperbilirubinemic rats (EHBRs), lacking Mrp2 due a genetic mutation, can only excrete MPAG to a limited degree in bile after intravenous administration of MPA, resulting in high MPAG plasma concentrations [13].

In this study, we aimed to elucidate the role of MRP2 in the interaction between MMF and CsA by repeating our previous pharmacokinetic study that was performed in wildtype rats [7], in transport-deficient (TR-) Wistar rats. TR- rats have a mutation in the *mrp2* gene which results in the absence of functional Mrp2 protein. Phenotypically, TR- rats are characterised by a defective hepatobiliary excretion of bilirubin glucuronides and other amphiphilic anions [12,14,15]. We show that in the absence of Mrp2, the previously described effects of CsA on MMF pharmacokinetics are no longer present, giving equal MPA and MPAG plasma concentrations as compared with rats treated with MMF and Tac. For the first time, these *in vivo* data provide evidence for the hypothesis that inhibition of MRP2 by CsA is the main mechanism responsible for the interaction between CsA and MPA.

MATERIALS AND METHODS

Animals

Adult male TR- Wistar rats (HRD-AMC Abcc2) were purchased from Harlan (Horst, the Netherlands). The animals were housed in microisolation cages (three per cage) and had free access to food and water. Rats were acclimated under a 12-hour light/dark cycle for two weeks before the start of the study. All rats were 12 weeks of age and weighed 250 grams at the start of the experiment.

Drug formulations

As placebo (hereafter called vehicle) we used Basis pro Suspension (Fagron Pharmaceuticals B.V., Nieuwerkerk a/d IJssel, the Netherlands) which consisted of 0.75 mg methylhydroxybenzoate, 0.20 mg propylhydroxybenzoate, 10.0 mg aluminiummagnesiumsilicilate, 10.0 mg carmellose sodium 500 mPas.s, 0.75 mg citric acid 1 *aq*, 263.0 mg sirupus simplex, and 783.30 mg purified water per mL. MMF powder (Cellcept®, Roche Bioscience, Palo Alto, Calif., USA) was suspended in vehicle for oral gavage every three days to produce a 2% solution which was stored at 4°C. CsA oral microemulsion formulation (100 mg/mL; Neoral®, Novartis Pharma AG, Basel, Switzerland) was freshly diluted in vehicle once daily to produce a 0.8% solution for oral gavage. Tac solution for intravenous injection (10 mg/mL; Prograf®, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan) was diluted in vehicle once daily to produce a 0.4% solution which was administered by oral gavage.

All drugs were kindly supplied by the manufacturers.

Study design

The study was a three-arm, two-period pharmacokinetic drug interaction study. Thirty adult, male TR- rats were allocated to three study groups (n=10 each). The possible drug interactions between MPA and CsA and between MPA and Tac were studied after CsA and Tac reached steady-state, and after single and multiple MMF doses. The drug dosages used were chosen on the basis of previous experience demonstrating their ability to prevent the occurrence of acute rejection after kidney transplantation in rats [7].

Vehicle group

After daily oral gavage with vehicle on days 0-6, MMF treatment (20 mg/kg bodyweight) was started on day 7 and was administered daily through day 14. Blood samples for MPA and MPAG pharmacokinetic analysis were collected during the 24 hours subsequent to dosing on day 7 (single dose pharmacokinetic profile). Additional MPA and MPAG 24-hour pharmacokinetic profiles (multiple dose) were determined subsequent to dosing on study day 14.

Cyclosporine group

From study day 0 through day 14, one group of rats was treated with 8 mg/kg bodyweight CsA daily. On day 7, co-administration of MMF (20mg/kg bodyweight) was started and continued through day 14. Blood samples for MPA and MPAG pharmacokinetic profiles were collected during 24 hours subsequent to dosing on study days 7 (single dose) and 14 (multiple dose). In addition, blood was collected at these time points for predose CsA concentration measurements.

Tacrolimus group

The dosing schedule and the schedule for the collection of samples for pharmacokinetic profiles was identical to those described for CsA. The rats received a daily Tac dose of 4 mg/kg bodyweight per day instead of CsA.

All drugs were given once daily by oral gavage at 9:00 am. When drugs were co-administered, they were given within 5 minutes. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes by tail bleeding under isoflurane (Rhodia Organique Fine Ltd., Bristol, UK) anaesthesia before and 0.5, 1, 2, 6, 12 and 24 hours after dosing. After collection, EDTA blood samples were immediately centrifuged at 11750 *g*, whereafter the plasma was frozen at –80 °C.

Ethics

The experimental protocol was approved by the Animal Experiments Committee of the Erasmus Medical Center under the national Experiments on Animals Act and adhered to the rules laid down in this national law that serves the implementation of "Guidelines on the protection of experimental animals" by the Council of Europe (1986), Directive 86/609/EC.

Quantification of plasma levels of the study drugs

CsA and Tac whole blood concentrations were determined with the Emit 2000 assay (Syva company, Dade Behring Inc., Cupertino, Calif., USA) on a Cobas Mira Plus analyzer (Roche Diagnostic Systems, Basel, Switzerland). Details on the sensitivity and reproducibility of the Emit assay in our laboratory were published previously [16]. Proficiency samples were obtained from the United Kingdom Quality Assessment Scheme (Dr Holt, St George's Hospital Medical School, London, United Kingdom).

MPA and MPAG in rat plasma were simultaneously measured with high-performance liquid chromatography (HPLC) according to the method described by Shipkova [17] with several modifications. The assay was validated for determination of MPA and MPAG in rat plasma according to FDA guidelines [18]. The limit of quantification was arbitrarily set at 0.25 mg/L for MPA and 2.5 mg/L for MPAG. The assay was found to be linear in a concentration range from 0.25 mg/L to 30 mg/L for MPA and from 2.5 mg/L to 100 mg/L for MPAG (correlation coefficient >0.99). In the same concentration range, the within-day CV ranged from 2.0% to 3.1% for MPA and from 1.5% to 3.9% for MPAG. The between-day CV ranged from 1.9% to 8.5% for MPA and from 3.0 to 6.9% for MPAG. The accuracy of the assay, defined as the percentage of recovery of MPA and MPAG from the control samples was for MPA in the range of 108-110% and for MPAG in the range of 98-113%.

Pharmacokinetic analysis

The concentration-time data were analysed using WinNonlin version 4.1 (Pharsight Corporation, Mountain View, Calif., USA). A noncompartmental model with extravascular input for plasma data was used to obtain estimates for MPA and MPAG maximum concentration (C_{max}), time to maximum concentration (T_{max}) and AUC. Since the AUC on study day seven was measured after the first MMF dose, $AUC_{0-\infty}$ was estimated. The decline of MPA or MPAG concentration between two subsequent time points that was most representative for elimination was used for extrapolation beyond 24 hours. On day 14 steady-state was assumed and AUC_{0-24} was calculated. AUCs were calculated by using the logarithmic trapezoidal rule.

Biochemistry

Before the start of the study and on study days 7 and 14, blood urea nitrogen, serum albumin and serum total bilirubin were determined on an ELAN analyzer (Eppendorf-Merck, Darmstadt, Germany) using the UV test/GIDH method, BCG method, and DPD methods, respectively (Merck Diagnostica, Darmstadt, Germany).

Statistical analysis

All data are expressed as means ± standard deviation unless otherwise stated. For comparisons between groups we used one-way ANOVA or Kruskal-Wallis test, as appropriate. Post-hoc analysis was performed using Bonferroni's test for multiple comparisons or using the Mann Whitney U test. For comparisons within groups, the paired t-test was used. P-values at $\alpha \leq 0.05$ were considered statistically significant. Statistical analysis was performed using SPSS for Windows version 11.5.0 (SPSS, Chicago, IL, USA).

RESULTS

Mycophenolic Acid and Glucuronidated Mycophenolic Acid Pharmacokinetics

A total of 30 MPA and MPAG pharmacokinetic profiles was obtained on study day 7 (single MMF dose). In all three treatment groups, the MPA concentration *vs.* time profiles were characterised by a first peak within the first hour followed by a second peak at 6-12 hours after drug administration, consistent with enterohepatic recirculation of MPA (Figures 1a and 2a).

On day 7, after the first MMF dose, the mean $AUC_{0-\infty}$ of MPA was significantly different between the three treatment groups: 32.0 ± 8.0 *vs.* 24.5 ± 6.1 *vs.* 21.8 ± 6.4 mg x h/L for the vehicle, CsA and Tac groups, respectively (p=0.007; Figure 2a and Table 1). This overall difference resulted from a significantly higher $AUC_{0-\infty}$ of MPA in the vehicle group as compared with the Tac group (p=0.008). When the MPA exposure was compared between the vehicle and the CsA groups a similar trend was observed, although this difference was not statistically significantly different (p=0.065). The $AUC_{0-\infty}$ of MPA in the CsA and Tac groups were not different (p=1.00).

After the first week, two rats in the Tac group died due to aspiration and therefore only 28 MPA and MPAG pharmacokinetic profiles were available for study day 14 (after multiple MMF doses). Again, we found an overall difference in MPA exposure between the different study groups (p=0.018) but there

was no difference in the AUC_{0-24} of MPA between rats receiving Tac or CsA: 28.1 ± 10.3 *vs.* 30.0 ± 13.3 mg x h/L, respectively (p=1.00; Figure 2b and Table 1). When the MPA exposure in the Tac and CsA groups was compared with the vehicle group, only the difference between the Tac and vehicle group was significant, although a similar trend was observed between the CsA and vehicle groups (p=0.033 and p=0.056, respectively; Figure 2b and Table 1).

Table 1 summarises the $AUC_{0-\infty}$ and AUC_{0-24} of MPAG values in the three different treatment groups at the two time points. Individual MPAG concentration *vs.* time profiles are depicted in Figure 1b. In line with our observations for MPA, the MPAG exposure was never significantly different between the Tac and CsA groups. However, on study day 7, there was an overall difference in MPAG exposure between the three groups which was caused by a significantly lower $AUC_{0-\infty}$ of MPAG in the vehicle group as compared with the Tac group but not the CsA group (overall p=0.022). On study day 14, this difference was no longer present, although the AUC_{0-24} of MPAG remained numerically highest in the rats treated with a calcineurin inhibitor (p=0.28; Table 1, Figure 3).

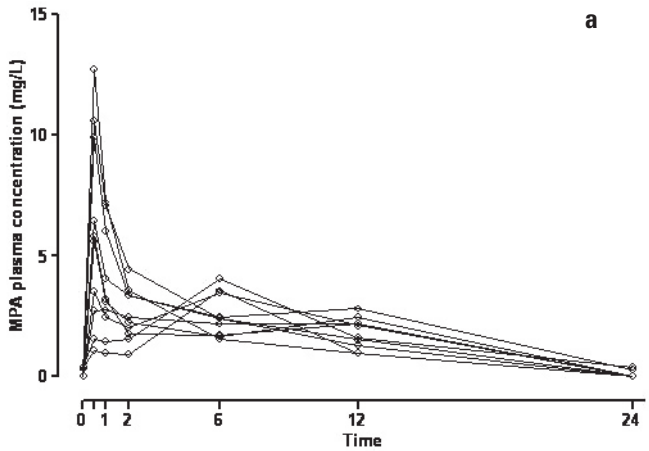
The MPA to MPAG-$AUC_{0-\infty}$ ratio was significantly different between the three treatment groups on study day 7: 0.10 ± 0.01 *vs.* 0.07 ± 0.02 *vs.* 0.05 ± 0.02 for the vehicle, CsA and Tac groups, respectively (p<0.001). The MPA to MPAG-AUC_{0-24} ratio on study day 14 was also significantly different between the three groups: 0.13 ± 0.01 *vs.* 0.08 ± 0.03 *vs.* 0.08 ± 0.03 for the vehicle, CsA and Tac groups, respectively (p=0.001). On study days 7 and 14, this difference was caused by a significantly higher MPA:MPAG ratio in the vehicle group as compared to either the CsA or the Tac group (p<0.001 and p=0.002, for study days 7 and 14, respectively), whereas the CsA and Tac groups did not differ significantly on study days 7 or 14 (p=0.17 and p=1.00, respectively)

Table 1 Pharmacokinetic data of MPA and MPAG in Mrp2 transport-deficient (TR-) rats treated with MMF in combination with either vehicle, cyclosporine (CsA) or tacrolimus (Tac) (n=10 in each group). Data represent the mean ± SD.

Study Day	Analyte	Parameter	Treatment Group		
			MMF + Vehicle	**MMF + CsA**	**MMF + Tac**
Day 7 (single dose)	MPA	$AUC_{0-\infty}$ (mg x h/L)	32.0 ± 8.0	24.5 ± 6.1	21.8 ± 6.4 [b]
		C_{max} (mg/L)	7.9 ± 4.9	4.0 ± 1.3 [a]	4.0 ± 2.2 [a]
		T_{max} * (h)	0.5 (0.5 - 6.0)	0.5 (0.5 - 12.0)	0.8 (0.5 - 24.0)
	MPAG	$AUC_{0-\infty}$ (mg x h/L)	324.4 ± 76.2	376.8 ± 87.2	422.3 ± 53.9 [a]
		C_{max} (mg/L)	38.3 ± 13.3	27.8 ± 7.0	30.8 ± 7.4
		T_{max} (h)	2.0 (0.5 - 12.0)	4.0 (1.0 - 24.0)	4.0 (1.0 - 12.0)
Day 14 (multiple dose)	MPA	AUC_{0-24} (mg x h/L)	41.7 ± 6.4	30.0 ± 13.3	28.1 ± 10.3 [a]
		C_{max} (mg/L)	6.5 ± 3.4	5.1 ± 4.3	3.2 ± 1.8 [a]
		T_{max} (h)	0.5 (0.5 - 6.0)	0.5 (0.5 - 2.0)	0.5 (0.5 - 2.0)
	MPAG	AUC_{0-24} (mg x h/L)	328.4 ± 30.1	365.0 ± 75.2	366.2 ± 58.3
		C_{max} (mg/L)	36.9 ± 7.9	33.0 ± 11.3	30.7 ± 7.9
		T_{max} (h)	2.0 (1.0 - 12.0)	7.0 (1.0 - 12.0)	12.0 (1.0 - 12.0)

* For T_{max} data represent the median (range)
[a] $p < 0.05$, significantly different from vehicle group
[b] $p < 0.01$, significantly different from vehicle group

Figure 1 Individual mycophenolic acid (A) and mycophenolic acid-glucuronide (B) pharmacokinetic profiles after once daily administration of 20 mg MMF/kg bodyweight to male Mrp2 transport-deficient (TR-) Wistar rats. Depicted are the concentration *versus* time profiles of the rats in the placebo group that were obtained on study day 14 (after multiple MMF dosing).



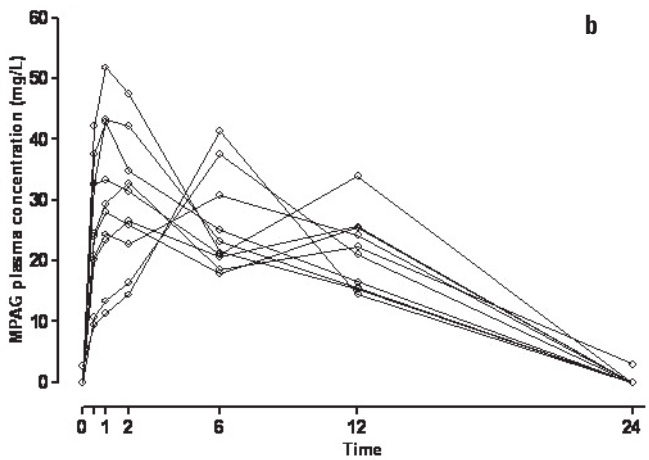


Figure 2 Mean (± SEM) mycophenolic acid (MPA) plasma concentrations in Mrp2 transport-deficient (TR-) rats after once daily administration of 20 mg MMF/kg bodyweight in combination with either vehicle (triangles), cyclosporine (solid circles) or tacrolimus (open circles)(n = 10 in each group). Depicted are the MPA pharmacokinetic profiles obtained on study day 7 (after a single MMF dose; panel 2A) and on day 14 (after multiple MMF doses; panel 2B).

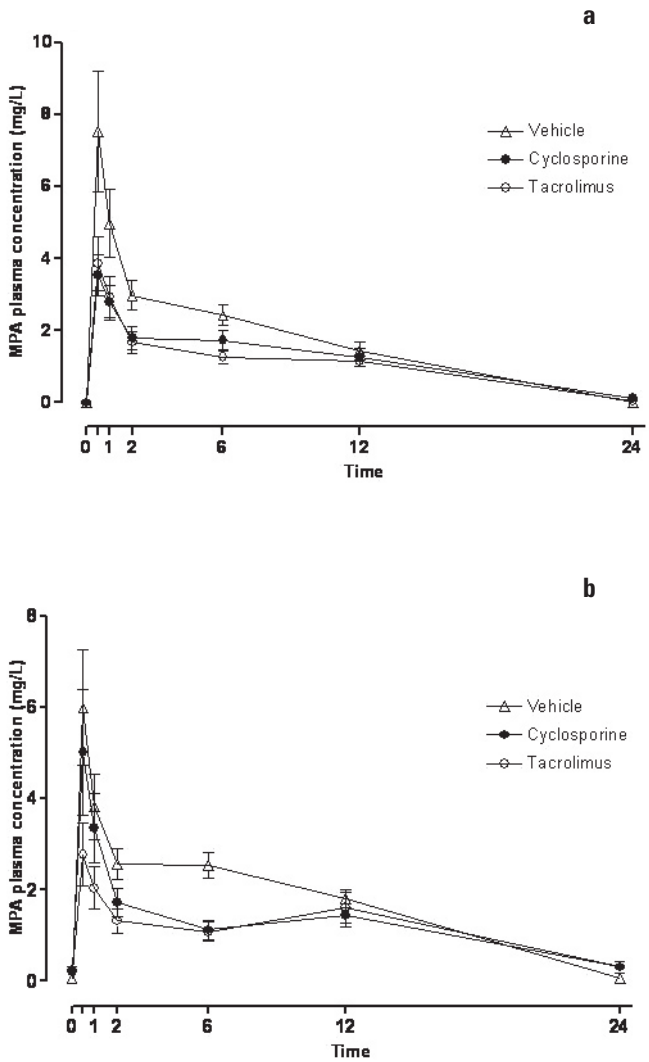
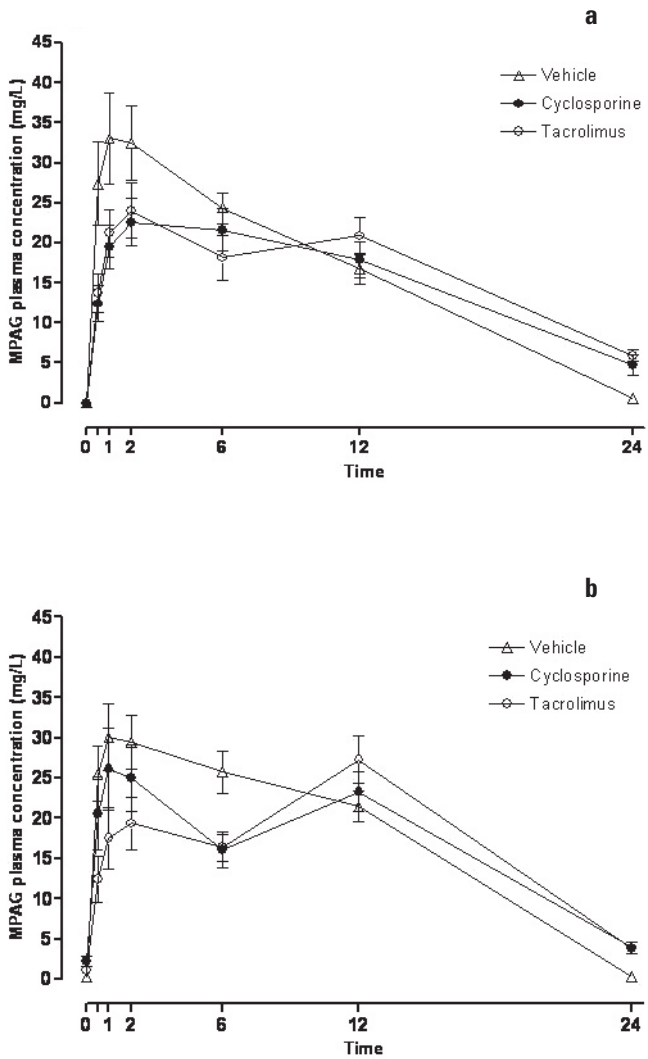


Figure 3 Mean (± SEM) mycophenolic acid glucuronide (MPAG) plasma concentrations in Mrp2 transport-deficient (TR-) rats after once daily administration of 20 mg MMF/kg bodyweight in combination with either vehicle (triangles), cyclosporine (solid circles) or tacrolimus (open circles)(n = 10 in each group). Depicted are the MPAG pharmacokinetic profiles obtained on study day 7 (after a single MMF dose; panel 3A) and on day 14 (after multiple MMF doses; panel 3B).



Cyclosporine and Tacrolimus Whole Blood Concentrations

The mean CsA predose concentrations at study days 7 and 14 were 456 ± 234 and 367 ± 137 ng/mL, respectively and were not significantly different (p=0.44). The Tac predose concentrations were also comparable between the two time points: 3.1 ± 1.7 *vs.* 2.3 ± 0.8 ng/mL for day 7 and 14, respectively (p=0.55).

Serum Chemistries

To exclude significant nephrotoxicity caused by CsA or Tac as a cause of possible differences in MPA and MPAG pharmacokinetics, we measured blood urea nitrogen concentrations at baseline and on study days 7 and 14. Throughout follow-up, the mean blood urea nitrogen was comparable between the three groups (Table 2). For serum albumin, there existed a significant overall difference between the three groups at study days 7 and 14 (overall $p<0.001$ and $p<0.01$, respectively; Table 2), which was caused by a lower serum albumin in the CsA group as compared with both the vehicle and Tac groups.

The serum total bilirubin concentrations at baseline were markedly elevated but not different between the three groups (p=0.22; Table 2). However, on study days 7 and 14, the serum total bilirubin concentration was significantly higher in the CsA and Tac groups as compared with the vehicle group (Table 2 and Figure 4). On study day 7, there also existed a difference in serum total bilirubin between the CsA and Tac group, but at the end of the study period, the bilirubin concentrations in the rats receiving a calcineurin inhibitor were comparable (Table 2 and Figure 4).

Figure 4 Mean serum total bilirubin concentrations (± SEM) in Mrp2 transport-deficient (TR-) rats after once daily administration of 20 mg MMF/kg bodyweight in combination with either vehicle (triangles), cyclosporine (solid circles) or tacrolimus (open circles).

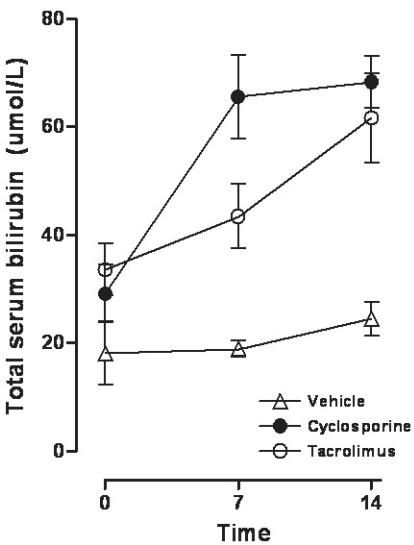


Table 2 Serum biochemistries of transport-deficient rats treated with MMF in combination with either vehicle, cyclosporine or tacrolimus (n=10 in each group). Data represent the mean ± SD.

		Vehicle	Cyclosporine	Tacrolimus
Albumin (g/L)	day 0	31.2 ± 4.0	31.4 ± 0.7	30.8 ± 2.4
	day 7	29.8 ± 1.8 [a]	27.6 ± 1.2	30.4 ± 1.2 [a]
	day 14	29.7 ± 2.1 [b]	27.4 ± 1.8	30.0 ± 1.4 [b]
Total bilirubin (µmol/L)	day 0	18.1 ± 10.2	29.1 ± 10.6	33.5 ± 9.6
	day 7	18.9 ± 4.6	65.5 ± 24.3 [c]	43.4 ± 18.7 [a, c]
	day 14	24.5 ± 10.0	68.3 ± 15.2 [c]	61.6 ± 23.3 [d]
Blood Urea Nitrogen (mmol/L)	day 0	8.8 ± 0.3	8.8 ± 0.3	9.3 ± 0.7
	day 7	8.4 ± 0.8	9.0 ± 0.7	8.9 ± 0.9
	day 14	7.0 ± 0.7	7.2 ± 0.9	7.3 ± 0.3

[a] $p < 0.001$, significantly different from cyclosporine group
[b] $p < 0.05$, significantly different from cyclosporine group
[c] $p < 0.001$, significantly different from vehicle group
[d] $p < 0.01$, significantly different from vehicle group

DISCUSSION

Several authors have shown that MPA exposure is significantly lower in CsA compared with Tac-based immunosuppressive regimens [3-6]. The reported difference is as high as 30-40% and is clinically relevant. As a consequence of this difference in MPA exposure, the optimal MMF maintenance dose in CsA-treated patients could be different from that in Tac-treated patients. Also, discontinuation of CsA treatment will lead to increased MPA exposure without a change in the MMF dose and can lead to the occurrence of new MMF-related side effects [4,19]. With the increasing interest in therapeutic drug monitoring of MMF therapy, the calcineurin inhibitor of choice is of high relevance.

In this study, we show that in the absence of the drug-transporting protein Mrp2, the pharmacokinetics of MPA and MPAG are comparable between rats receiving either CsA or Tac as co-medication. In addition, the ratio of the MPA to MPAG-AUC of TR- rats treated with MMF and vehicle (which averaged around 1:9) was comparable to the MPA:MPAG ratio that we observed previously in wildtype rats treated with MMF and CsA [7]. In the current study, identical CsA and Tac doses on a bodyweight basis were used, resulting in similar predose concentrations. For the first time, these results demonstrate *in vivo*, that Mrp2 is the transporter mainly responsible for the excretion of MPAG into bile and that inhibition of Mrp2 by CsA is the mechanism underlying the interaction between CsA and MPA. Because the difference in MPA exposure between CsA and Tac co-administration appears to be present in both rats and transplanted patients, the results of the present experimental study are relevant for the human situation. There only appears to be a quantitative difference between both species. In humans, the contribution of enterohepatic recirculation to total MPA exposure averages around 40%, whereas in rats it can account for as much as 70%.

Further evidence for the role of MRP2 in the excretion of MPAG comes from the results of Sallustio [20] who perfused isolated rat livers obtained from normal and TR- rats, with MPA and measured MPA and MPAG concentrations in both perfusate and bile. In normal rats, more than 90% of the administered MPA dose was recovered as MPAG in bile and no MPAG was present in the perfusate. In marked contrast, less than 1% of the MPA dose was recovered as MPAG from the bile of TR- rats, and around 80% was recovered as MPAG in the perfusate. Importantly, glucuronidation of MPA to

MPAG appeared to be comparable between normal and Mrp2-deficient rats [20]. A markedly reduced excretion of MPAG into bile was also demonstrated in EHBRs (that lack Mrp2) after intravenous MPA administration [13]. However, in this report, the interaction between MMF and calcineurin inhibitors was only directly studied in normal, but not in Mrp2-deficient animals. Moreover, all drugs were administered intravenously and were not in steady state [13]. For the current study, we compared the results of the Mrp2-deficient Wistar rat with those from our previous experiment in wildtype Lewis rats. Wildtype Wistar rats have been shown to metabolise several drugs by glucuronidation in the liver, with subsequent biliary excretion and enterohepatic recirculation. For diclofenac and valproic acid it was recently demonstrated that biliary excretion of their respective metabolites is mediated by Mrp2 [21,22]. This shows that, similar to normal Lewis rats, wildtype Wistar rats possess a functional Mrp2-mediated enterohepatic recirculation of glucuronidated substances and obviates the need for a control group of wildtype Wistar rats.

Interestingly, in this study, we observed marked differences in the MPA and MPAG plasma concentrations between the vehicle group and the groups receiving a calcineurin inhibitor: The mean MPA exposure in the vehicle group was higher compared with rats receiving a calcineurin inhibitor, while the opposite was true for MPAG exposure. There are several possible explanations for this finding. First, in TR- rats, the absence of Mrp2 may be partly compensated for by (the induction of) other organic anion transporters. Alternative drug-transporting enzymes that could theoretically provide an escape mechanism for MPAG excretion into bile may include the breast cancer resistance protein (or ABCG2), the bile salt export pump (or ABCB11), and others [23-25]. Calcineurin inhibitors are known to inhibit a variety of these drug-transporters [26-29]. P-glycoprotein (or ABCB1) is a less likely candidate as this transporter is not known to transport glucuronidated substances. If alternative MPAG-elimination pathways are operational in the liver of the TR- rat, then their blockade by CsA or Tac would theoretically lead to an increased MPAG exposure and, as less MPAG is available for enterohepatic recirculation, to a decreased MPA exposure compared with rats receiving vehicle. The observation that bilirubin concentrations were highest in rats who were treated with calcineurin inhibitors suggests that inhibition of alternative MPAG-excretory mechanisms may indeed have occurred in our experiment.

Another, not mutually exclusive, escape mechanism would be that of an increased secretion of MPAG into sinusoidal blood in the calcineurin inhibitor groups. It may be speculated that (induction of) MRP3/Mrp3 is involved. Mrp3 has a high affinity for glucuronide substrates and is known to be overexpressed in the EHBR [30,31], as well as in humans with the Dubin-Johnson syndrome who are deficient for MRP2 [32]. MRP3/Mrp3 is located on the basolateral membrane of the hepatocyte and mediates the transport of organic anions from the hepatocyte into sinusoidal blood [32-34]. Xiong [35] reported an increased basolateral egress of acetaminophen-glucuronide in the TR- rat, suggesting up-regulation of an organic anion transporter on the basolateral membrane of Mrp2-deficient rat livers.

A second mechanism that may have contributed to the differences in MPA/MPAG exposure between the vehicle and CsA/Tac-treated rats, could be an increased MPA clearance in the latter group. The observation that the MPA peak concentrations were lower in the rats treated with a calcineurin inhibitor is suggestive in this respect. High bilirubin or elevated MPAG plasma concentrations may result in a decreased binding of MPA to plasma albumin, thus increasing the free fraction of MPA, resulting in a more rapid conjugation of MPA to MPAG [2,36,37]. Alternatively, a lowering of serum albumin, as we observed in the CsA treated rats, could also have increased the free fraction of MPA. However, the reduction of serum albumin was limited and we feel that this mechanism is unlikely to have influenced the disposition of MPA or MPAG in our experiment to a significant degree [37].

Finally, the observation that co-administration of MMF with CsA or Tac, lowered MPA peak concentrations compared with rats in the vehicle group, may also suggest that treatment with calcineurin inhibitors decreased the absorption of MPA. It has previously been demonstrated that treatment with CsA is associated with overexpression of P-glycoprotein in parenchymal cells of human kidney allografts [38]. This indicates that CsA may induce its own detoxification by upregulation of P-glycoprotein expression in the kidney. Although Koziolek [38] did not investigate the expression levels of P-glycoprotein in the small intestine, increased P-glycoprotein levels would in theory result in a lowering of the oral bioavailability of P-glycoprotein substrates.

CONCLUSION

In conclusion, our data provide evidence that the pharmacokinetic interaction between CsA and MPA is caused by inhibition of Mrp2 by CsA.

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Chapter 2.6

Pharmacokinetic Modeling of Plasma Protein Binding of Mycophenolic Acid in Renal Transplant Recipients

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ABSTRACT

Renal function and plasma albumin level have been shown to correlate with the clearance (CL) of total mycophenolic acid (MPA). This population pharmacokinetic study aimed at developing a semi-mechanistic model to elucidate the mechanism by which renal function and plasma albumin affect the disposition of MPA.

774 total MPA (MPA_t), 479 unbound MPA (MPA_u), and 772 total MPA glucuronide metabolite ($MPAG_t$) plasma concentrations were available from 88 renal transplant recipients on day 11 and 140 after transplantation. Data were analysed using non-linear mixed effects modeling.

Time profiles of MPA_u and $MPAG_t$ concentrations were adequately described by two 2-compartment pharmacokinetic models with a link between the central compartments. MPA_t concentrations were modeled using: $[MPA_t] = [MPA_u] + [MPA_u]*\theta_{protein\ binding}$, with $[MPA_u]*\theta_{protein\ binding}$ representing the bound MPA concentration. $\theta_{protein\ binding}$, and therefore $[MPA_t]$, was significantly correlated with creatinine clearance (CrCl), plasma albumin level and the $MPAG_t$ concentration (all $p<0.001$). These correlations can be described in terms of changes in f_u, because the fraction unbound $(f_u) = [MPA_u] / [MPA_t] = 1 / (1 + \theta_{protein\ binding})$: a reduction of CrCl from 60 to 25 mL/min correlated with an increase in f_u from 2.7% to 3.5%, accumulation of $MPAG_t$ concentrations from 50 to 150 mg/L correlated with an increase in f_u from 2.8% to 3.7%, and a decrease in plasma albumin level from 40 to 30 g/L correlated with an increases of f_u from 2.6% to 3.5%. No significant correlations were detected between MPA_u CL and plasma albumin level or CrCl.

The model shows that low CrCl, low plasma albumin levels, and high MPAG concentrations decrease total MPA exposure by affecting MPA binding to albumin.

INTRODUCTION

Mycophenolic acid (MPA) is the immunosuppressive active compound of the prodrug mycophenolate mofetil, which is widely used to prevent acute rejection after kidney, liver and heart transplantation [1]. The pharmacokinetics of MPA are characterised by its extensive binding to plasma albumin with an unbound fraction (f_u) of less than 3% [2]. MPA is believed to undergo restrictive clearance, because only the unbound MPA fraction is presumed to be available for elimination from the body through metabolism to the phenolic MPA glucuronide metabolite (MPAG) in liver, kidney, and intestinal mucosa [3-5]. Furthermore, unbound MPA is thought to be responsible for immunosuppressive activity [6]. MPAG has a high protein binding (82%) as well, and has been shown to displace MPA from its albumin binding sites at high concentrations *in vitro*. *In vivo* high MPAG concentrations occur during renal insufficiency [2,6].

Previous studies showed that total MPA exposure is significantly influenced by renal function and plasma albumin level [7-10]. As a hypothesis for the underlying mechanism, it was proposed that low plasma albumin levels and accumulation of MPAG decrease the binding of MPA to albumin. The subsequent increase of MPA f_u produces an increased total MPA clearance (CL), in line with the restrictive clearance concept for MPA [5,11]. Consequently, total MPA concentrations will decrease, while unbound MPA exposure is expected to be unaffected. In contrast with this theory, previous pharmacokinetic studies have found that low plasma albumin levels, impaired renal function or high MPAG levels result in an increase of unbound MPA $AUC_{0\text{-}12}$ [12-14]. Furthermore, two other studies showed both increased unbound MPA exposure and decreased total MPA exposure in a situation of renal dysfunction [9,15]. Consequently, the mechanism of the effect of renal function and plasma albumin level on total and unbound MPA exposure is uncertain, as well as if and how the dose of mycophenolate mofetil should be adjusted in cases of impaired renal function or low plasma albumin levels.

The aim of this study was to develop a semi-mechanistic population pharmacokinetic model for total and unbound MPA plasma concentrations, as well as for total MPAG plasma concentrations to study the mechanism by which renal function and plasma albumin level affect the pharmacokinetics of MPA. This knowledge may aid in optimising the mycophenolate mofetil dose in situations of impaired renal function and low plasma albumin levels.

PATIENTS AND METHODS

Total MPA (MPA_t) and unbound MPA (MPA_u) as well as total MPAG ($MPAG_t$) plasma concentration-time data from 88 renal transplant recipients who participated in a randomised concentration controlled trial (RCCT) were analysed retrospectively. A detailed description of the RCCT has been published previously [16,17]. Briefly, the aim of the RCCT was to investigate the relationship between exposure to total MPA and clinical outcome (incidence of acute rejection and adverse events). Patients aged 18 years or older were randomly assigned to three MPA_t AUC target groups: low (AUC 16.1 mg*h/L), intermediate (AUC 32.2 mg*h/L) or high (AUC 60.6 mg*h/L). Starting mycophenolate mofetil doses for these groups were respectively, 450 mg twice daily, 950 mg twice daily and 1700 mg twice daily, and doses were adjusted based on repetitive measurements of MPA AUC. All patients received cyclosporine and corticosteroids as concomitant immunosuppressive therapy according to routine practice.

Sampling Procedure

In the RCCT, MPA_t and $MPAG_t$ concentration-time samples were collected from 150 renal transplant patients on day 3, 7, 11, 21, 28, 56, 84, 112 and 140 after renal transplantation. Plasma samples for description of full 12-hour concentration-time profiles were collected on days 3, 7 and 11 after transplantation. Sample times were predose and 0.33, 0.66, 1.25, 2, 6, 8 and 12 hours after oral intake of mycophenolate mofetil. For the remaining occasions, serial plasma samples were collected according to a limited sampling strategy. Sample times were predose and 0.33, 0.66, 1.25, and 2 hours after mycophenolate mofetil administration [17]. Patients were required to fast overnight prior to dose administration and for the first 2 hours of the profile after taking the medication. On each sampling day, routine laboratory tests were performed. From a subset of the patients (n=88) MPA_u concentrations were measured on one or two of the nine occasions, which were nominally day 11 and/or day 140. Pharmacokinetic data for MPA_t and $MPAG_t$ plasma concentrations were included in the present analysis when MPA_u data were available as well. Patient characteristics and biochemical parameters are summarised in table 1. MPA_t and $MPAG_t$ was measured using a validated high-performance liquid chromatography (HPLC) method described elsewhere [18]. The lower limit of quantification was 0.1 mg/L for MPA_t and 4.0 mg/L for $MPAG_t$. MPA_u was measured with a validated ultrafiltration procedure, described previously [6].

Table 1 Patient demographics and biochemical parameters on the two sampling occasions after renal transplantation (results presented as median (range))

Characteristics	Missing data (%)	Day 11 (range: 7-19)	Day 140 (range: 111-148)
Gender (n)			
Male	0	52	12
Female	0	31	16
Race (n)			
Caucasian	0	79	24
Black	0	0	1
Other	0	4	3
Diabetes mellitus (n)	0	9	2
DGF (n)	0	10	-
Use of antacids (n)	0	18	1
Age (years)	0	51 (19 - 70)	51 (21 - 69)
Body weight (kg)	7	67 (40 - 99)	69 (46 - 98)
Serum creatinine (µmol/L)	0	152 (66 - 659)	122 (78 - 244)
Creatinine clearance (mL/min)	7	45 (8 - 107)	55 (33 - 95)
Plasma albumin (g/L)	13	35 (26 - 43)	40 (27 - 49)
Serum ALT (U/L)	9	22 (3 - 178)	9 (4 - 52)
Serum AST (U/L)	11	14 (3 – 50)	16 (5 – 110)
Serum total bilirubin (mg/dL)	12	0.6 (0.2 - 1.8)	0.6 (0.2 - 2.0)
Haemoglobin (g/dL)	0	9.5 (6.9 - 14)	11 (8.9 - 14)
Prednisone daily dose (mg)	3	25 (15 - 1275)	10 (5 - 20)
Cyclosporine daily dose (mg)	4	550 (250 -1125)	250 (150 - 500)
Cyclosporine predose concentration (ng/mL)	2	265 (21 - 1166)	132 (59 - 294)
MMF dose (mg twice daily)	0	1300 (350 - 2200)	975 (300 - 2200)

In total, data were collected from 88 renal transplant recipients. Data were available from 83 patients on day 11 after renal transplantation, and from 28 patients on day 140. 25 patients were sampled on both occasions. Two patients had their only available sampling occasion between day 19 and 111 (day 55 and 57), their demographic data are not included in the table. n = number of renal transplant recipients; DGF = delayed graft function; MMF = mycophenolate mofetil. Normal values as used at Erasmus MC, Rotterdam, The Netherlands for creatinine: 65-115 µmol/L for males and 55-90 µmol/L for females, for plasma albumin level: 35-50 g/L, for serum ALT: <41 U/L for males and <31 U/L for females, for serum AST: <37 U/L for males and <31 U/L for females, for serum total bilirubin: <1 mg/dL, for haemoglobin: 13.8-16.9 g/dL for males and 12.1-15.3 mg/dL for females.

Pharmacokinetic modeling

All data were analysed simultaneously using the nonlinear mixed effects modeling software program NONMEM (double precision, Version V, level 1.1, GloboMax LLC, Hanover, USA). Because NONMEM estimated pharmacokinetic parameters for MPA, MMF doses were converted to the equivalent MPA content by multiplying MMF dose by 0.739. MPA_t and MPA_u concentrations, as well as $MPAG_t$ concentrations and albumin levels were converted to units of mmol/L (M_w MPA = 320.34 g/mol, M_w MPAG = 496.472 g/mol, M_w albumin = 69000 g/mol). Data were logarithmically transformed (natural logarithm) and the first order (FO) estimation method was used throughout the entire model building process, because of the high computational intensity of the first order conditional estimate method (FOCE).

The data were analysed using a three step approach. During the first step, a compartmental model was developed, in which the pharmacokinetics of MPA_u as well as the protein binding of MPA were described, including between- and within-patient variability. For MPA_u, several compartmental models were evaluated. In addition, it was investigated whether absorption was best described by a first- or zero-order process, and with or without a lag time. Pharmacokinetic parameters for MPA_u were estimated in terms of CL, central and peripheral volumes of distribution (V1, and V2 and V3 respectively) and intercompartmental clearances (Q2, Q3). Since bioavailability (F) could not be quantified, apparent oral values of MPA_u CL, Q and V were estimated, these values are the ratios of MPA_u CL/F, Q/F and V/F respectively. Models were parameterised using the ADVAN6 routine for general nonlinear models in NONMEM. Between- and within-patient variability in all estimated parameters was characterised using exponential models [19]. For example, MPA_u CL for the i^{th} individual on the j^{th} occasion (CL_{ij}) was estimated using equation 1:

$$MPA_u\ CL_{ij}\ (L/h) = \theta_{pop} * \exp(\eta_i + \kappa_j) \qquad \text{(Eq. 1)}$$

where θ_{pop} represents the population value for MPA_u CL. η_i and κ_j represent the between- and within-patient random effect, respectively, with mean 0 and variance ω^2 and π^2.

MPA_t was modeled as follows:

$$[MPA_t]\ (mmol/L) = [MPA_u] + [MPA_u] * \theta_{protein\ binding} \qquad \text{(Eq. 2)}$$

where $[MPA_t]$ is the MPA_t concentration, $[MPA_u]$ is the MPA_u concentration, and $\theta_{protein\ binding}$ is a factor that correlates to the number of unoccupied albumin binding places. $[MPA_u] * \theta_{protein\ binding}$ represents the bound MPA concentration [20]. According to equation 2, f_u equals:

$$f_u = \frac{[MPA_u]}{[MPA_t]} = \frac{[MPA_u]}{[MPA_u] + [MPA_u] * \theta_{protein\ binding}} = \frac{1}{1 + \theta_{protein\ binding}} \qquad \text{(Eq. 3)}$$

Equation 2 and 3 represent linear protein binding. Nonlinear and saturable protein binding were evaluated as well.

The difference between the k^{th} observed MPA_u or MPA_t concentration of the i^{th} individual (C_{obsik}) and its corresponding model predicted MPA_u or MPA_t concentration (C_{predik}) was modeled as additive to the log of the observed MPA_u or MPA_t concentration (equation 4) [21]:

$$\ln C_{obsik} = \ln C_{predik} + \varepsilon_{ik} \qquad \text{(Eq. 4)}$$

where ε is the residual random error with mean 0 and variance σ^2. The result of step 1 was a basic model for unbound and total MPA.

In step 2 of the analysis, the influence of demographic and pathophysiological factors on the pharmacokinetic parameters as well as on MPA protein binding were investigated. The tested covariates included age, gender, weight, diabetic status, delayed graft function (defined as the need for dialysis during the first two weeks after renal transplantation), plasma albumin level, liver enzymes AST and ALT, bilirubin, hemoglobin, mycophenolate mofetil dose, cyclosporin daily dose, cyclosporine predose level, corticosteroid dose, the use of antacids, and creatinine clearance (CrCl) as a measure for renal function, calculated using the Cockroft and Gault formula (table 1) [22]. First, covariates were introduced separately in the basic model for unbound and total MPA and tested for their significance

in a univariate analysis. Continuous covariates, for instance weight, were modeled in an exponential manner, which allows for estimation of nonlinear relationships [23] (equation 5):

$$MPA_u\ CL_{ij}\ (L/h) = \theta_{pop} * (Weight/67)^{\theta_{Weight}} \quad \text{(Eq. 5)}$$

in which θ_{pop} is the MPA_u CL in an individual with a weight of 67 kg (median of the population) and θ_{Weight} is an exponent determining the shape of the relationship. Categorical variables, like for example gender, were modeled as shown in equation 6:

$$MPA_u\ CL_{ij}\ (L/h) = \theta_{pop} * \theta^{gender} \quad \text{(Eq. 6)}$$

where θ_{pop} is the population value for MPA_u CL in females (gender = 0) and θ^{gender} is the fractional change of MPA_u CL in males (gender = 1). 0 - 13% of the covariate data were missing (table 1). Missing data were imputed with the population median value of the sampling occasion on which the covariate data item was missing (day 11 or 140 after transplantation). The statistical significance of the correlation between the covariate and the pharmacokinetic parameter was assessed by using the log-likelihood test, in which the difference between the minimum value of objective function (OFV) generated by NONMEM for two hierarchical models is determined [24]. When inclusion of a covariate caused a decrease of the OFV of >10.8 units ($p<0.001$, 1 degree of freedom), the covariate was considered to be statistically significant. Furthermore, a reduction in between- or within-patient variability was used as a criterion for covariate selection, as well as the biological plausibility of a relationship. All covariates selected during the univariate analysis were included in the intermediate model. Subsequently, covariates were excluded separately in a backward elimination procedure. If the elimination of a covariate significantly worsened the fit of the model (increase of OFV>10.8), the covariate remained in the model. The result of step 2 was a covariate model for unbound and total MPA.

In Step 3 of the analysis, $MPAG_t$ concentrations were included in the population model. Since unbound MPAG concentration were not available in the present analysis, the central compartment for $MPAG_t$ was linked directly with the central compartment for MPA_u. Models with one and two compartments were evaluated for $MPAG_t$. Besides MPAG, MPA is also metabolised to other compounds, like the acylglucuronide metabolite [3,4]. Because metabolite concentrations other than MPAG were not available in this study, the fraction of MPA metabolised to MPAG (F_M) could not be quantified. Consequently, the pharmacokinetic parameters of $MPAG_t$ represent the ratios of $MPAG_t$ CL/F_M, Q/F_M, and V/F_M. The difference between observed and model predicted $MPAG_t$ concentrations (residual variability) was modeled as additive to the log of the observed $MPAG_t$ concentration (equation 4). The influence of $MPAG_t$ concentrations on MPA protein binding was modeled as shown in equation 7, which is derived from equation 2:

$$[MPA_t]\ (mmol/L) = [MPA_u] + [MPA_u] * (\theta_{protein\ binding} * (1 - \theta_{MPAG} * ([MPAG_t] - 0.13))) \quad \text{(Eq. 7)}$$

in which θ_{MPAG} is the linear fractional change of $\theta_{protein\ binding}$ per mmol/L change in $MPAG_t$ concentration with the latter centered on the median value of 0.13 mmol/L. Further relationships between covariates and pharmacokinetic parameters were investigated as described above. The result was the final model.

The adequacy of a model was evaluated using the precision of the parameter estimates and goodness-of-fit plots [25]. Goodness-of-fit plots were generated in Xpose (version 3.010), an S-PLUS based (Version 6.2, professional edition, Seattle, USA) modeling aid.

RESULTS

113 concentration-time profiles were available form 88 patients containing 479 MPA_u, 774 MPA_t and 772 $MPAG_t$ plasma concentrations. Eighty three concentration-time curves were obtained between day 7 and 19 after transplantation, 1 profile on day 55, 1 profile on day 57 and 28 profiles were taken between day 111 and 148 after transplantation. A concentration-time curve of MPA_u, MPA_t, and $MPAG_t$ from a representative patient is shown in figure 1. The median number of MPA_u samples drawn during a concentration-time curve was 2 (range: 1-8). The observed relationship between MPA_t and MPA_u concentrations is depicted in figure 2a; the calculated f_u versus time is shown in figure 2b. Both plots indicate linear protein binding over the whole concentration range. Figure 2 shows one patient with high MPA f_u values (>14%). This patient had a CrCl of 33 mL/min and a plasma albumin level of 27 g/L.

During step 1 of the analysis, MPA_u concentration data were fitted to several compartmental models. A two compartment model with time lagged zero order absorption and with first order elimination adequately fitted the data. The relationship between MPA_t and MPA_u concentration was best described by a linear model, and models for nonlinear, saturable, protein binding did not improve the fit. Between-patient variability could be estimated for the duration of the zero-order absorption, MPA_u V1, MPA_u CL and $\theta_{protein\ binding}$; corresponding values were 100%, 86%, 36% and 22%, respectively. Within-patient variability could be estimated for MPA_u CL and was found to be 27%. Introduction of within-patient variability in MPA_u CL reduced the residual variability in both MPA_u concentration and MPA_t concentration from 0.47 to 0.45. Parameter estimates of the basic model for unbound and total MPA are summarised in table 2.

During step 2, relationships between patient factors and pharmacokinetic parameters were investigated. The covariate analysis yielded an intermediate model with the following significant correlations: CrCl, plasma albumin level, age, and weight for the duration of absorption; CrCl, plasma albumin level, weight, gender, and the use of antacids for MPA_u V1; cyclosporine daily dose and corticosteroid dose for MPA_u CL; CrCl and plasma albumin level for $\theta_{protein\ binding}$. Of note, the effect of plasma albumin level on MPA protein binding was best modeled by multiplying this covariate with $\theta_{protein\ binding}$ (table 2), indicating that the number of unoccupied binding places correlated with plasma albumin level. No significant correlations were detected between MPA_u CL and plasma albumin level and CrCl. During the backward elimination procedure, exclusion of the relationships between CrCl and MPA_u V1, cyclosporine daily dose and MPA_u CL, CrCl and $\theta_{protein\ binding}$, and plasma albumin level and $\theta_{protein\ binding}$ resulted in an increase of the OFV of at least 12 units, and, therefore, remained in the model. The parameter estimates of the covariate model for unbound and total MPA after the backward elimination procedure are presented in table 2.

In step 3, the covariate model for unbound and total MPA was extended with the modeling of total MPAG data. The $MPAG_t$ concentration-time data fitted a two compartment model with first order elimination well. Between-patient variability could be estimated for $MPAG_t$ CL, and was found to be 27%. Addition of an effect of CrCl on $MPAG_t$ CL improved the fit: OFV decreased by 287 units and the estimate of residual variability for $MPAG_t$ concentrations decreased from 0.24 to 0.18. Introduction of a correlation between $MPAG_t$ concentrations and $\theta_{protein\ binding}$, according to equation 7, resulted in a decrease of OFV of 16 units, and the estimate for between-patient variability in $\theta_{protein\ binding}$ decreased from 16% to 12%. Also nonlinear models were tested for the effect of $MPAG_t$ concentration on $\theta_{protein\ binding}$, but these did not perform better than the model in equation 7. A second backward elimination procedure was preformed using the selected covariates during the third step of the analysis and the retained covariates from the second step. All covariates remained in the model as OFV increased at least 12 units upon exclusion. The only relationship which did not result in an increase of OFV >10.8

upon exclusion was the correlation between CrCl and MPA_u V1. Therefore, this relationship was excluded from the model.

Thus, the final model consisted of two 2-compartment models with a central and peripheral compartment for both MPA_u and $MPAG_t$ with a link between the central compartments (figure 3), and with MPA_t modeled according to equation 2. Parameter estimates of the final model are presented in table 2. Population MPA_u CL and $MPAG_t$ CL in the final model were described by equation 8 and 9, respectively, both derived from equation 5:

$$MPA_u\ CL\ (L/h) = 1070 * (\text{cyclosporine daily dose (mg)}/490)^{0.43} \quad \text{(Eq. 8)}$$

$$MPAG_t\ CL\ (L/h) = 1.67 * (CrCl\ (mL/min)/47)^{0.83} \quad \text{(Eq. 9)}$$

MPA_u CL typically decreased from 1166 to 869 L/h when cyclosporine daily dose decreased from 600 to 300 mg ($p<0.001$, equation 8). When CrCl decreased from 60 to 25 mL/min, $MPAG_t$ CL decreased from 2.0 to 1.0 L/h (equation 9).

MPA f_u could be described with:

$$f_u = \frac{1}{1 + (64 * \text{plasma albumin level} * (CrCl/47)^{0.29} * (1 - 1.28 * (MPAG\ C_t - 0.13)))} \quad \text{(Eq. 10)}$$

in which 64 is the population estimate for $\theta_{\text{protein binding}}$, corresponding to a population MPA f_u of 2.9% (equation 3), when plasma albumin level is 36 g/L (0.52 mmol/L). Equation 10 described a typical increase in f_u from 2.7% to 3.5% when CrCl decreased from 60 to 25 mL/min (figure 4a). Furthermore, accumulation of $MPAG_t$ concentration from 50 to 150 mg/L (= 0.10 to 0.30 mmol/L) correlated with an increase in f_u from 2.8% to 3.7% (figure 4b), and a change in plasma albumin level from 40 to 30 g/L (= 0.60 to 0.43 mmol/L) correlated with an increases of f_u from 2.6% to 3.5% (figure 4c). When the described changes in CrCl, $MPAG_t$ concentration and plasma albumin level occur simultaneously, equation 10 predicts an increase of f_u from 2.3% to 5.3%. The identified correlations with $\theta_{\text{protein binding}}$ imply that a change in CrCl, $MPAG_t$ concentration and plasma albumin level produces a change in MPA_t concentration as $\theta_{\text{protein binding}}$ directly affects MPA_t concentration according to equation 2.

CrCl, $MPAG_t$ concentration and plasma albumin level explained 45% of the between-patient variability in $\theta_{\text{protein binding}}$, as the estimate for this parameter dropped from 22% in the basic model for unbound and total MPA, to 12% in the final model. Furthermore, cyclosporine daily dose could explain 31% of the between-patient, and 26% of the within-patient variability in MPA_u CL. Goodness-of-fit plots are given in figures 5 to 7. Figures 5 to 7 show a symmetrical and random pattern around the line of identity (figures 5 and 6) or the line y=0 (figure 7). Because 95% of weighed residuals fall between -2 and 2, and because the parameters in the final model are estimated with an acceptable coefficient of variation of $\leq 44\%$ for fixed effects, and $\leq 88\%$ for random effects, the model is believed to describe the data adequately.

Table 2 Parameter estimates for the basic model for unbound and total MPA, the covariate model for unbound and total MPA, and the final model with their coefficients of variation (CV)

Parameter	Basic model for unbound and total MPA	Covariate model for unbound and total MPA	Final model
	Estimate (CV%)		
Minimum OFV	-82	-227	-1109
PK parameters			
Duration of absoprtion (zero-order) (h)	0.66 (22%)	0.89 (10%)	0.88 (7%)
T_{lag} (h)	0.09 (62%)	0.10 (29%)	0.10 (41%)
MPA_u V1 (L)	3700 (17%)	2460 (13%)	2990 (27%)
MPA_u CL (L/h)	877 (8%)	1060 (6%)	1070 (6%)
MPA_u V2 (L)	36700 (22%)	6750 (34%)	6240 (26%)
MPA_u Q (L/h)	1030 (13%)	1160 (13%)	1210 (13%)
$\theta_{protein\ binding}$	31 (4%)	62 (3%)	64 (3%)
$MPAG_t$ V3 (L)	-	-	6.5 (23%)
$MPAG_t$ CL (L/h)	-	-	1.7 (3%)
$MPAG_t$ V4 (L)	-	-	9.1 (17%)
$MPAG_t$ Q (L/h)	-	-	11 (44%)
Between-patient variability			
Duration of absoprtion (zero-order) (%)	100 (29%)	64 (37%)	84 (39%)
MPA_u V1 (%)	86 (49%)	114(24%)	91 (30%)
MPA_u CL (%)	36 (38%)	25 (26%)	25 (32%)
$MPAG_t$ CL (%)	-	-	27 (22%)
$\theta_{protein\ binding}$ (%)	22 (60%)	16 (58%)	12 (88%)
Within-patient variability			
MPA_u CL (%)	27 (33%)	19 (36%)	20 (33%)
Residual variability			
Additive error MPA C_u (mmol/L)*	0.45 (6%)	0.41 (5%)	0.42 (6%)
Additive error MPA C_t (mmol/L)*	0.45 (6%)	0.43 (6%)	0.44 (6%)
Additive error MPAG C_t (mmol/L)*	-	-	0.18 (15%)
Covariate effects			
CsA dose on MPA_u CL	-	0.53 (14%)	0.43 (18%)
CrCl on MPA_u CL	-	-1.22 (19%)	-
CrCl on $\theta_{protein\ binding}$	-	0.39 (23%)	0.29 (34%)
MPAG Ct on $\theta_{protein\ binding}$	-	-	-1.28 (21%)
CrCl on $MPAG_t$ CL	-	-	0.83 (15%)

*Residual variability is on a natural logarithmic-scale as data were logarithmically transformed. CrCl = creatinine clearance, CsA = cyclosporine, MPA = mycophenolic acid, MPA C_u = unbound MPA concentration, MPA C_t = total MPA concentration, MPA_u V1 = unbound MPA central distribution volume, MPA_u CL = unbound MPA clearance, MPA_u V2 = unbound MPA peripheral distribution volume, MPA_u Q = unbound MPA intercompartment clearance, T_{lag} = lag time, MPAG = glucuronide metabolite of MPA, MPAG C_t = total MPAG concentration, $MPAG_t$ V3 = total MPAG central distribution volume, $MPAG_t$ CL = total MPAG clearance, $MPAG_t$ V2 = total MPAG peripheral distribution volume, $MPAG_t$ Q = total MPAG intercompartment clearance, OFV = objective function.

Figure 1 Concentration-time profiles of unbound mycophenolic acid (circles), total MPA (squares), and total glucuronide metabolite of MPA (triangles) for a representative patient on day 11 after renal transplantation. The administered dose was 650 mg mycophenolate mofetil (MMF), creatinine clearance was 45 mL/min, plasma albumin level was 35 g/L (0.51 mmol/L) and cyclosporine daily dose was 800 mg.

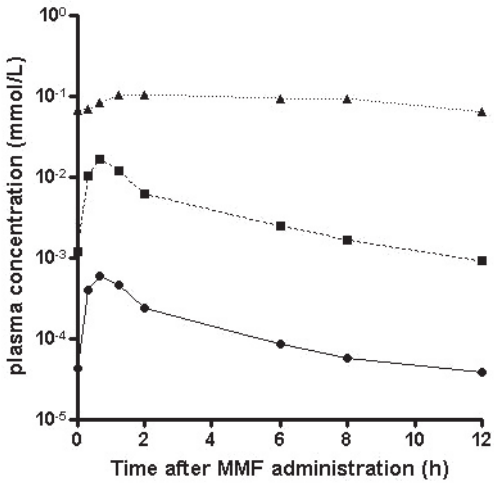


Figure 2 Correlation between a) unbound mycophenolic acid (MPA_u) plasma concentrations and total mycophenolic acid (MPA_t) plasma concentrations, and b) protocol sample time and mycophenolic acid unbound fraction (f_u). Data are from 113 concentration-time curves distributed over 88 adult renal transplant recipients. The small solid horizontal lines represent the median f_u for each protocol sampling time. The high MPA f_u values in a and b (>14%, closed circles) are from one patient who had a CrCl of 33 mL/min and a plasma albumin level of 27 g/L (= 0.39 mmol/L).

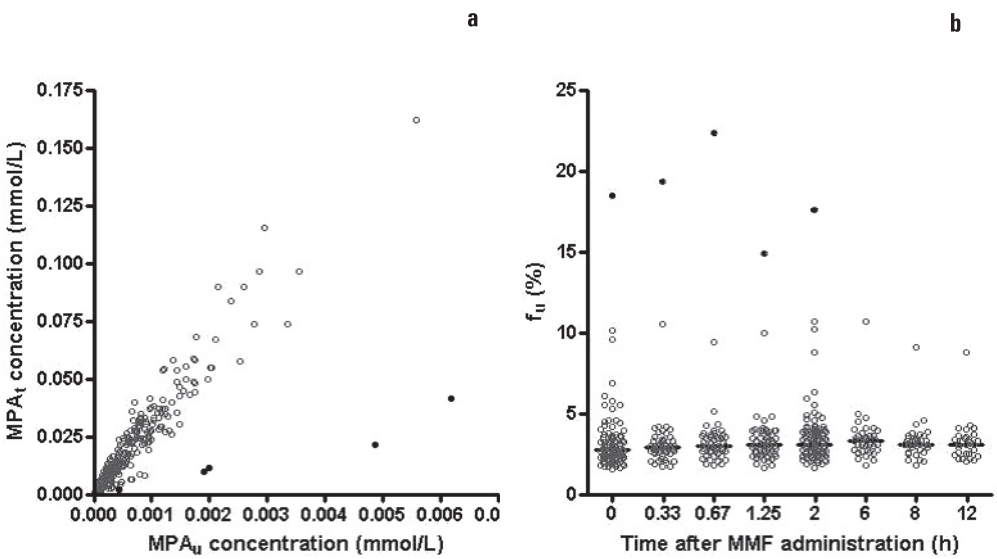


Figure 3 Schematic representation of the final population pharmacokinetic model. A 2 compartment model was used for both unbound MPA (MPA_u) plasma concentrations and total MPAG ($MPAG_t$) plasma concentrations with a link between the central compartments. The dotted arrow and compartment represent the protein binding and bound concentrations of MPA, respectively. Bound MPA was modeled as $[MPA_u] * \theta_{protein\ binding}$. Total MPA ($MPA_t$) plasma concentration was modeled using $[MPA_t] = [MPA_u] + [MPA_u] * \theta_{protein\ binding}$. CL = clearance, MMF = mycophenolate mofetil, Q = intercompartment clearance.

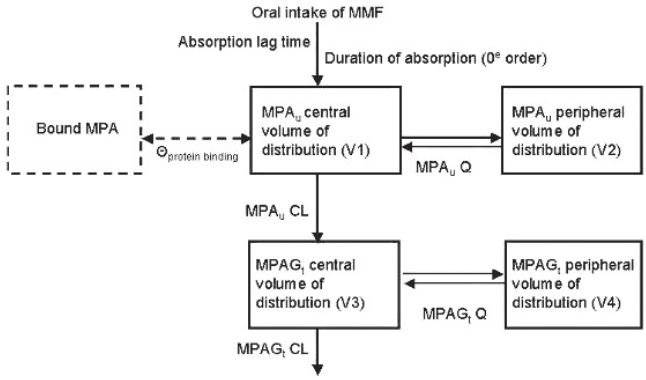


Figure 4 a) F_u versus creatinine clearance (CrCl). The solid line represents the estimated relationship for a typical patient with a plasma albumin level of 36 g/L (= 0.52 mmol/L), and a total plasma concentration of the glucuronide metabolite of MPA (MPAG) of 65 mg/L according to equation 10. b) F_u versus albumin level. The solid line represents the estimated relationship for a typical patient with a CrCl of 47 mL/min, and a total MPAG plasma concentration of 65 mg/L according to equation 10. c) F_u versus total MPAG plasma concentration. The solid line represents the estimated relationship for a typical patient with a CrCl of 47 mL/min, and a plasma albumin level of 36 g/L (= 0.52 mmol/L) according to equation 10.

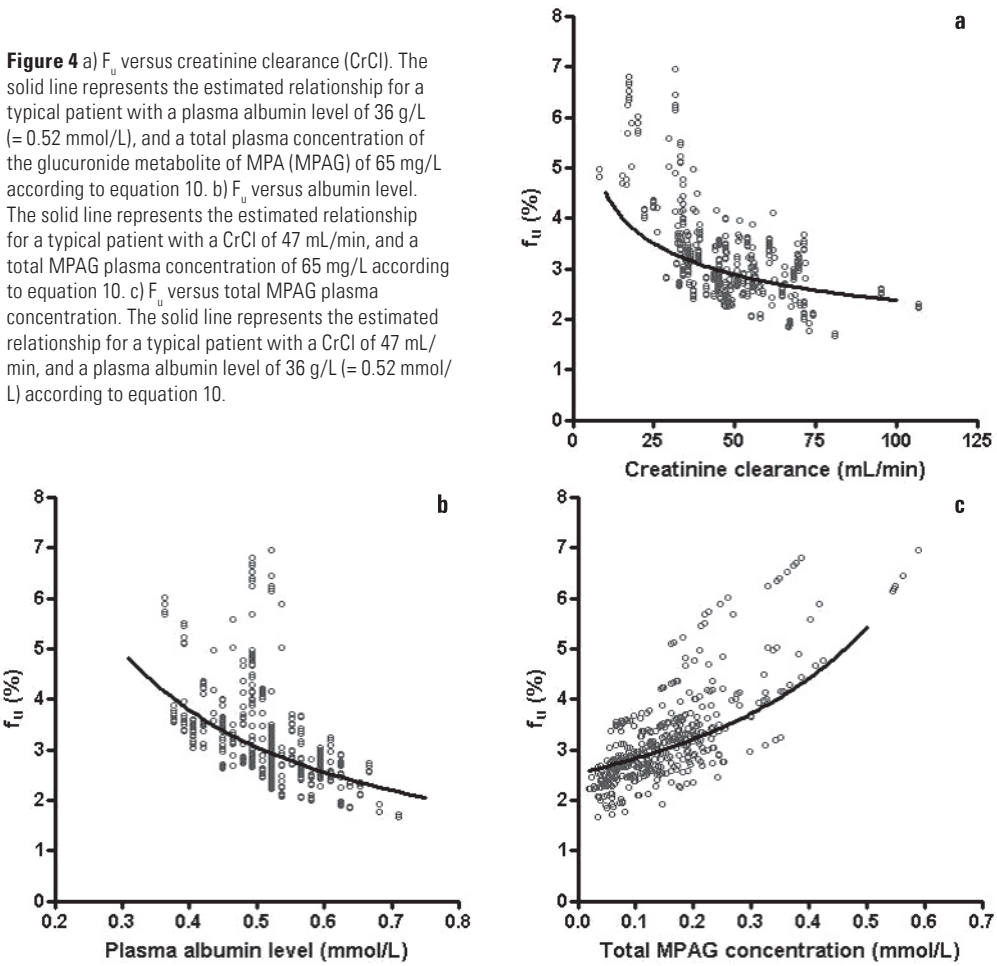


Figure 5 Population predicted plasma concentration versus observed plasma concentration for the final model for a) unbound mycophenolic acid (MPA) concentration, b) total MPA concentration, and c) total concentration of the glucuronide metabolite of MPA (MPAG). The solid lines represent the line of identity.

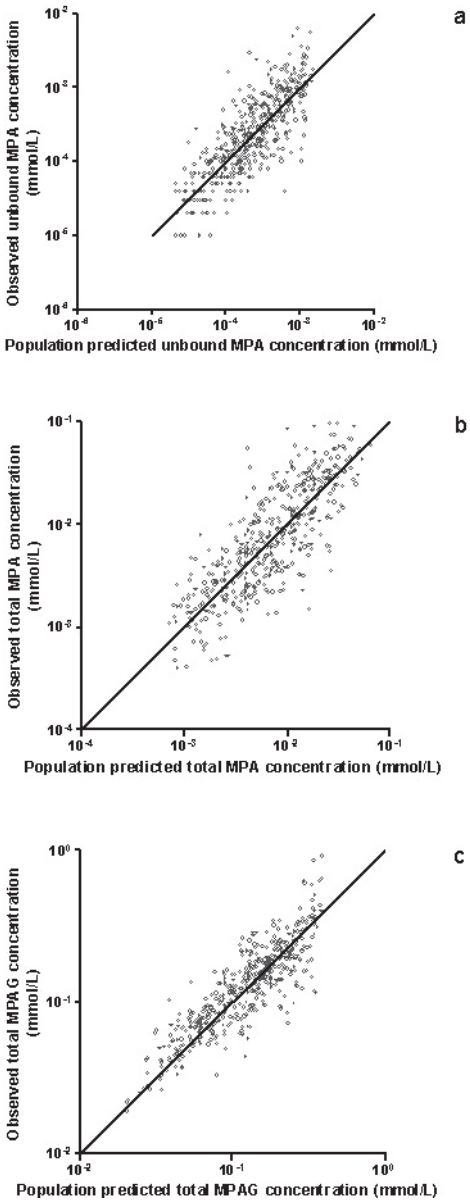


Figure 6 Individually Bayesian predicted plasma concentration versus observed plasma concentration for the final model for a) unbound mycophenolic acid (MPA) concentration, b) total MPA concentration, and c) total concentration of the glucuronide metabolite of MPA (MPAG). The solid lines represent the line of identity.

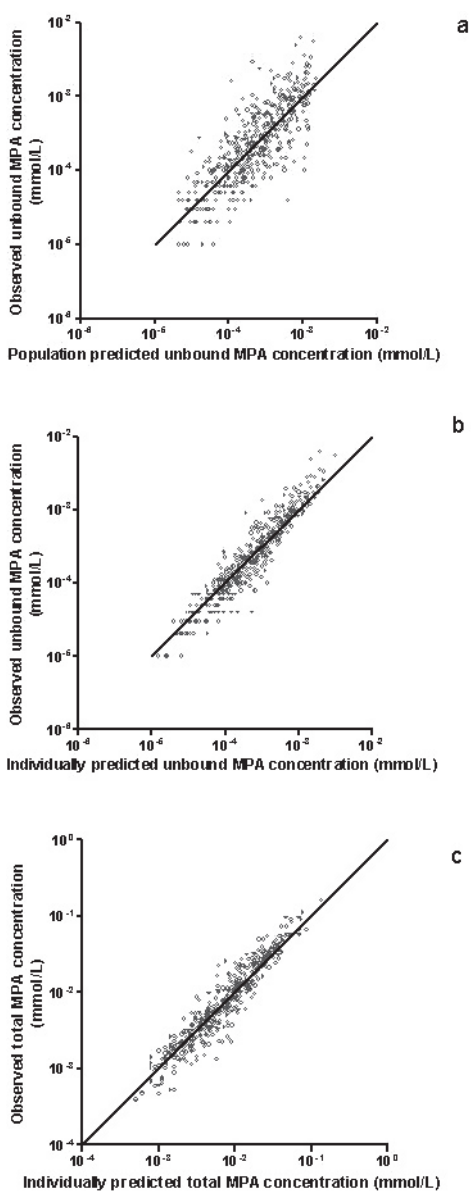
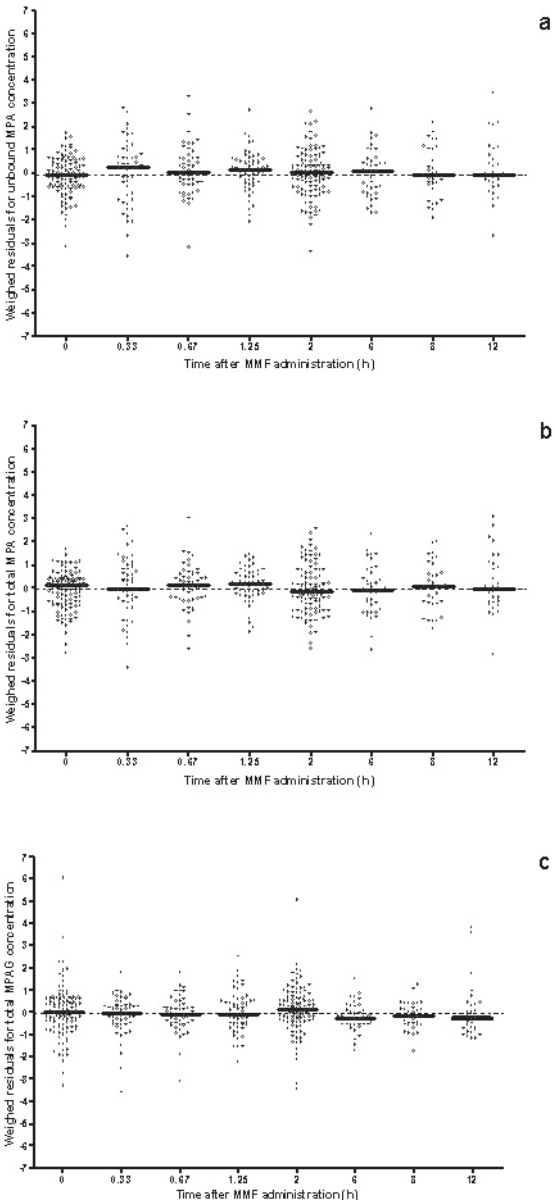


Figure 7 Protocol sample time versus weighed residuals for the final model for a) unbound mycophenolic acid (MPA) concentration, b) total MPA concentration, and c) total concentration of the glucuronide metabolite of MPA (MPAG). The dotted lines represent the line y = 0 and the small solid horizontal lines represent the median weighed residual for each protocol sampling time.



DISCUSSION

A significant effect of renal function and/or plasma albumin level on the pharmacokinetics of MPA in renal transplant recipients has been shown in many studies [7-10]. However, conflicting hypotheses exist about the underlying mechanism of the effects, and about the need for mycophenolate mofetil dose adjustments in situations of impaired renal function or low albumin levels.

The developed semi-mechanistic model supports the hypothesis, proposed by Nowak and Shaw [6,9], that low CrCl, low plasma albumin levels, and high MPAG concentrations affect exposure to total MPA through MPA protein binding. In the present analysis, statistically significant relationships were found between CrCl, total MPAG concentrations, and plasma albumin level and $\theta_{protein\ binding}$. These relationships imply that a decrease in CrCl or in plasma albumin level, or an increase in total MPAG concentrations correlate with an increase of MPA f_u (figure 4a to c). A change in f_u (i.e. $\theta_{protein\ binding}$) will subsequently lead to a change in total MPA exposure according to equation 2 and 3. Given that f_u is constant during a dosing interval (figure 2b), it can be calculated that total MPA $AUC_{0\text{-}12}$ will be halved when f_u is doubled. No effect could be shown of CrCl or plasma albumin level on MPA_u CL, indicating that unbound MPA concentrations are unaffected. These results are in accordance with the theory for a restrictively cleared drug [5].

The relationship between total MPAG concentrations and $\theta_{protein\ binding}$ in the final model provides *in vivo* evidence that MPAG displaces MPA from its albumin binding sites, as has been shown in a previous *in vitro* study [6]. Besides total MPAG concentrations, also CrCl was significantly correlated with $\theta_{protein\ binding}$. A priori it was expected that a model accounting for the effect of total MPAG concentrations on $\theta_{protein\ binding}$ would account for the total effect of CrCl. Nevertheless, the backward elimination procedure showed that both total MPAG concentrations and CrCl had an independent effect on $\theta_{protein\ binding}$ in the final model. The interpretation may be that renal function does not only affect MPA protein binding as a result of accumulation of total MPAG, but that also other effects of impaired renal function influence MPA binding to albumin. Possible factors that accompany poor renal function and that may alter MPA binding, may be metabolic acidosis and uremia [6,9].

Cyclosporine daily dose was significantly correlated with MPA_u CL in the final model. The likely mechanism for this relationship is cyclosporine mediated inhibition of the multidrug resistance protein 2 (MRP2) [26]. Normally, MPA undergoes enterohepatic recirculation: MRP2 excretes MPAG into bile, whereafter MPAG is deglucuronidated in the gut by the intestinal flora to form MPA, which is reabsorbed from the colon. The enterohepatic recirculation has been shown to be interrupted when MRP2 is inhibited by cyclosporine [26,27]. This leads to decreased exposure to total and unbound MPA, which is reflected in the present model by an increased MPA_u CL with higher doses of cyclosporine.

The inhibition of the enterohepatic recirculation by cyclosporine also offers a likely explanation why the final model showed an adequate fit, without accounting for the recycling of MPA. All patients in this study were concurrently treated with cyclosporine, and concentration-time profiles showed only a moderate contribution of enterohepatic recirculation. The weighed residuals versus time plot of the final model (figure 7) showed no trends indicating the lack of modeling enterohepatic recirculation.

Importantly, the results from this study are only valid for albumin levels between 25 and 49 g/L, CrCl above 8 mL/min and MPAG concentrations up to 450 mg/L. The model showed that in this range of albumin and MPAG concentrations, binding of MPA is linear (figure 2a, equation 2) and probably not saturated. With a plasma albumin level below 25 g/L, a CrCl below 8 mL/min or MPAG concentrations above 450 mg/L, MPA binding may become saturated. This could in theory lead to an increase in f_u and consequently a disproportional increase of unbound MPA concentrations. The reported increased unbound MPA $AUC_{0\text{-}12}$ values in previous studies and case reports may be explained along those lines, as these studies included patients with lower plasma albumin levels, lower CrCl values, and higher MPAG concentrations than the present analysis [12-14,32-34].

From a clinical point of view, it is not easy to make sound mycophenolate mofetil dose recommendations based on the results of the present study. In a situation of impaired renal function, high MPAG concentrations, or low plasma albumin level, the decreased total MPA exposure does not adequately reflect the unchanged unbound MPA exposure. The latter is regarded to be the pharmacologically active moiety and the unbound MPA level has been shown to correlate with the risk for leukopenia and infection [28,29]. From this perspective, dose adjustments based on total MPA exposure are not appropriate, and eventually, unbound MPA concentration should be measured to determine whether dose adjustments are indicated [30]. On the other hand, although a relationship between unbound MPA exposure and the risk for acute rejection would be obvious, it has not been shown yet in renal transplant recipients. A relationship between total MPA exposure ($AUC_{0\text{-}12}$ or predose concentration) and the risk for acute rejection does exist [16,17,31]. As a result, patients with poor renal function or low plasma albumin levels, and accompanying low total MPA exposure, are at increased risk for acute rejection. This would mean that mycophenolate mofetil dose adjustments according to measurement of total MPA exposure are indicated.

A consensus between these two seemingly contrasting conclusions may be that patients with impaired renal function or low albumin levels, in whom low total MPA concentrations have been observed, are candidates for mycophenlate mofetil dose increases to minimise the risk for acute rejection. The dose increase will also increase unbound MPA exposure. Therefore, the neutrophil count in these patients should be closely monitored, as there may be a potential increased risk for leukopenia and infections [28,29]. Of course, in patients with an already low neutrophil count, the risk between acute rejection and infection should be carefully balanced. In such a situation, measurement of unbound MPA concentrations may aid in the decision to adjust the mycophenolate mofetil dose, in which unbound MPA $AUC_{0\text{-}12} > 0.4$ mg*h/L is expected to increase the risk for haematologic side effects. This cut-off value has been established in a pediatric study in the early posttransplant phase [28], and such cut-off values are lacking for the adult renal transplant population. More information is needed about the relationship between unbound MPA exposure and the risk for rejection and side effects before more clear mycophenolate mofetil dosing recommendations can be made.

The current final model can be regarded as a semi-mechanistic model, as it does not fully reveal the albumin binding characteristics of MPA, and its displacement by MPAG. In the present analysis, it was attempted to fit the data to more mechanistic models, in which separate compartments were defined for unbound and bound MPA, as well as for unbound and bound MPAG. Such models require quantification of the protein binding characteristics of MPA and MPAG to physiologically model displacement of MPA from its albumin binding sites by MPAG. However, in the absence of unbound MPAG concentrations, it unfortunately appeared that the total MPAG concentration data did not contain enough information to ensure successful convergence of these models. Although the current final model is not a purely mechanistic model, it does show how CrCl, plasma albumin level and MPAG concentrations affect the pharmacokinetics of MPA. Therefore, the developed semi-mechanistic model is suitable to meet the aim of the present study.

CONCLUSION

In conclusion, the developed semi-mechanistic model supports the hypothesis that low CrCl, low plasma albumin levels, and high MPAG concentrations decrease total MPA exposure by affecting MPA binding to albumin in renal transplant recipients. No effect was observed on unbound MPA concentrations as no significant correlations were detected between MPA_u CL and plasma albumin level or CrCl.

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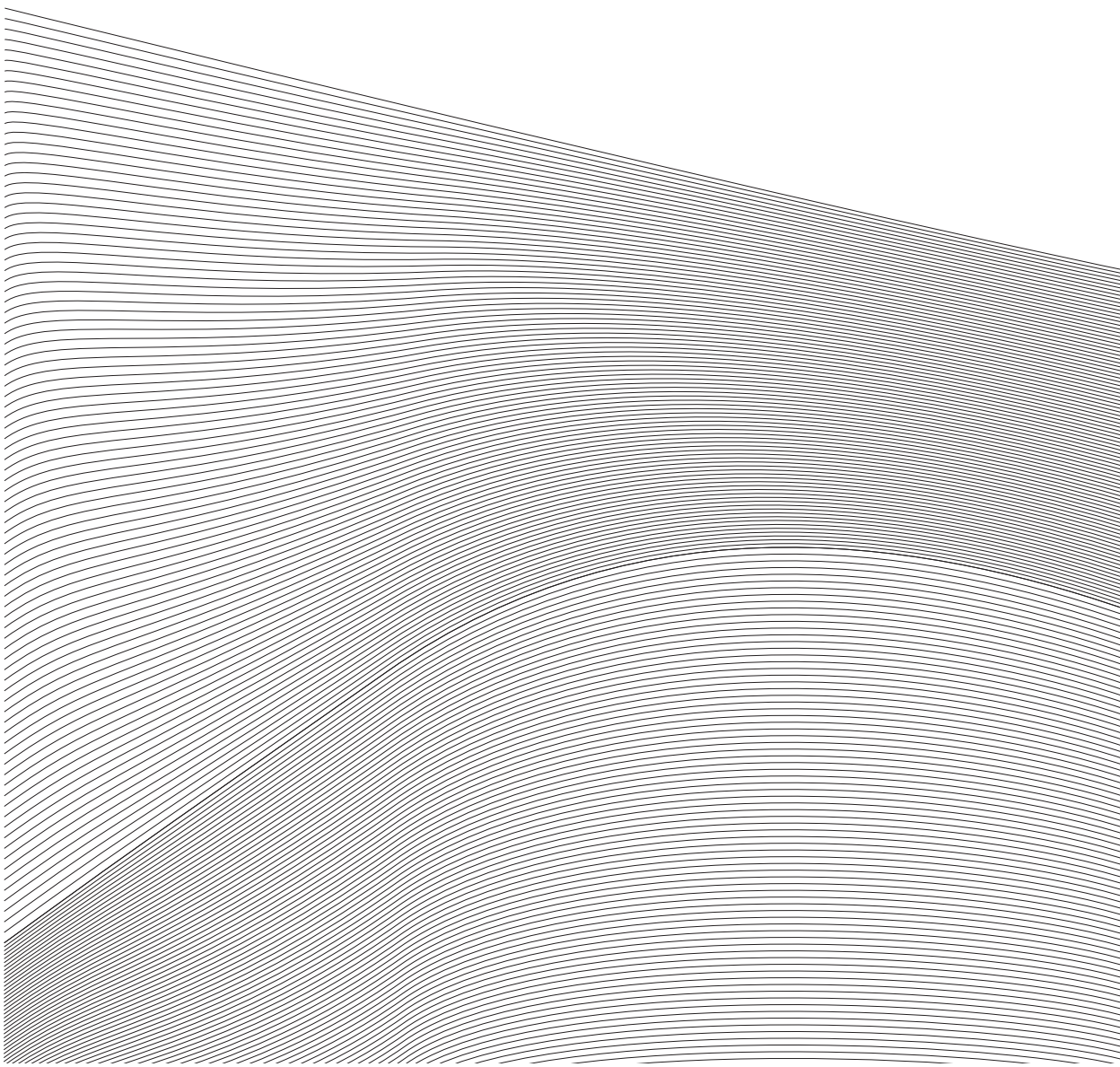
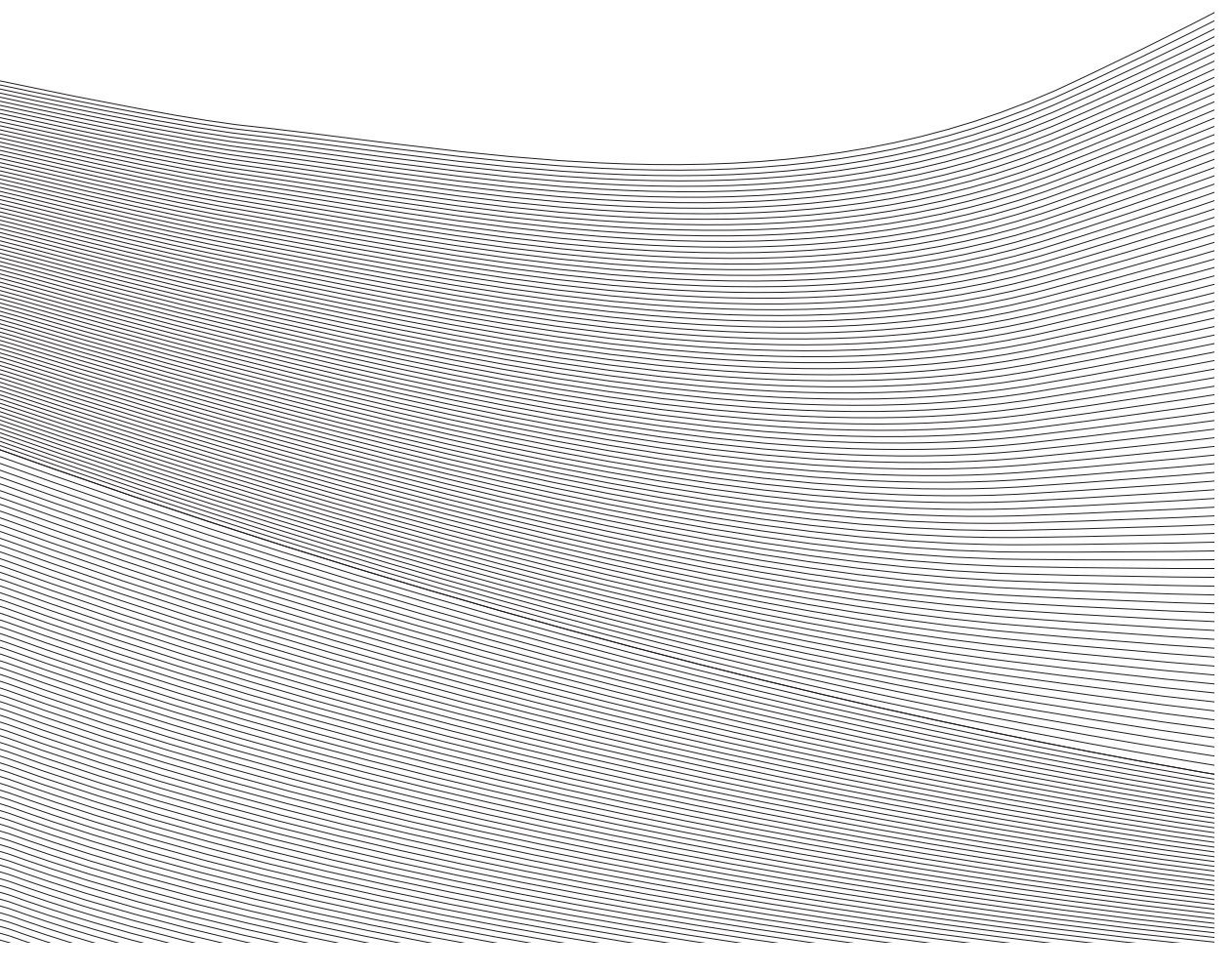


Chapter 3

INTRODUCING THERAPEUTIC DRUG MONITORING OF MYCOPHENOLIC ACID



Chapter 3.1

Predicting the Usefulness of Therapeutic Drug Monitoring of Mycophenolic Acid: A Computer Simulation

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ABSTRACT

The usefulness of therapeutic drug monitoring (TDM) of mycophenolate mofetil (MMF) was investigated with a computer simulation model. For a fixed-dose (FD) and a concentration-controlled (CC) MMF dosing regimen exposure to mycophenolic acid (MPA) was compared.

A nonlinear mixed-effects model (NONMEM) for MPA based on extensive pharmacokinetic data from 140 renal transplant recipients, who all used cyclosporine and corticosteroids as maintenance immunosuppressive therapy provided Bayesian estimates for MPA oral clearance on 9 occasions during the first 24 weeks after transplantation. In 45 of these patients, the estimates for MPA oral clearance were used to calculate values for the area under the curve (AUC) of MPA. In the CC group, MMF doses were adjusted based on the calculated AUC, targeting at an AUC level of 45 mg*h/L. In the FD group, MMF doses were fixed to 1000 mg.

On day 7 after transplantation significantly more AUC values were on target (AUC range 30-60 mg*h/L) in the CC group than in the FD group: 76% versus 13% respectively, $p<0.001$. To accomplish this, a doubling of MMF dose was necessary in more than half of the patients after the AUC assessment on day 3 after transplantation. Between-patient variability (BPV) in AUC (average CV% for all occasions) was reduced in the CC regimen: 23% versus 44% in the FD group.

By using TDM, adequate MPA exposure appears to be obtained more rapidly and BPV in exposure is reduced. To reach target AUC levels as soon as possible in this cyclosporine treated population, it appears that larger MMF doses as currently recommended are necessary in the first month after transplantation.

INTRODUCTION

Mycophenolate mofetil (MMF) is a prodrug of mycophenolic acid (MPA) and is widely used to prevent acute rejection of solid organ transplants. Its immunosuppressive activity arises from the reversible and noncompetitive inhibition of inosine monophosphate dehydrogenase (IMPDH), which leads to antiproliferative effects on T- and B-lymphocytes [1,2]. Prospective studies have established that the risk for acute graft rejection in both adult and paediatric renal transplant recipients is lower when MPA exposure, in terms of area under the plasma concentration versus time curve (AUC), is higher [3-5]. Several studies analysed the pharmacokinetics of MPA in renal transplant patients and showed a large between- and within-patient variability in MPA exposure as well as an increasing MPA AUC during the first months after transplantation [4,6,7]. Furthermore, effects of concomitant immunosuppressive therapy, as with cyclosporine (CsA), on MPA AUC have been reported [8,9]. Based on this knowledge a debate about the potential contribution of therapeutic drug monitoring (TDM) for MMF dose individualisation has emerged. Reducing between-patient variability in exposure by increasing MMF dose in individuals with low MPA exposure, and decreasing dose in those with high MPA levels, could potentially increase overall efficacy and reduce drug related toxicity. The current consensus is that MPA AUC is the parameter of choice, assessed by a limited sampling strategy for practical reasons, and that the MPA AUC should be targeted at 30-60 mg*h/L for at least the first 6 months after transplantation when MPA is combined with CsA [10]. Despite the available evidence of correlations between MPA plasma concentrations and likelihood of developing acute rejection, TDM of MPA is not applied on a routine basis. Before a prospective randomised trial is done comparing a concentration-controlled and a fixed dose MMF regimen, a computer simulation can be used to gain more insight in this issue. Trial simulations are known to give an impression about the outcomes of (planned) pharmacokinetic

studies and are increasingly being used to choose the design of a clinical trial that will best meet the study objectives [11-14]. This study aims at predicting the usefulness of TDM of MPA by simulating pharmacokinetic profiles for renal transplant patients receiving a fixed MMF dose (FD) and for patients receiving a pharmacokinetic guided MMF dose [concentration-controlled (CC)].

PATIENTS AND METHODS

Patients and sampling procedure

Concentration-time data from renal transplant patients who participated in a Randomised Concentration-Controlled Trial (RCCT) were analysed retrospectively. A detailed description of the methods and results of the RCCT was published elsewhere [3,4]. Briefly, the goal of the RCCT was to investigate the relationship between exposure to MPA (AUC, pre-dose concentration, maximum concentration) and outcome (risk for acute rejection, side effects). Adult patients were randomly assigned to three target MPA AUC groups: low (AUC: 16.1 mg*h/L, starting MMF dose 450 mg BID), intermediate (AUC: 32.2 mg*h/L, starting MMF dose 950 mg BID) or high (AUC: 60.6 mg*h/L, starting MMF dose 1700 mg BID). All patients received CsA and corticosteroids as concomitant immunosuppressive therapy.

Concentration-time samples were collected on 9 occasions: days 3, 7, 11, 21, 28, 56, 84, 112 and 140 after transplantation. Plasma samples for description of full 12-h concentration-time profiles on the first 3 sampling occasions were taken at pre-dose, 0.33, 0.66, 1.25, 2, 6, 8 and 12 hours after oral intake of MMF. For the remaining 6 occasions serial plasma samples were taken according to a limited sampling strategy at pre-dose, 0.33, 0.66, 1.25, and 2 hours post-dose [4]. Samples were analysed using a validated HPLC method described elsewhere [15]. The lower limit of quantification was 0.1 mg/L.

Computer simulation

A 2-compartment population pharmacokinetic model with time-lagged first-order absorption for MPA has been developed with the aid of the nonlinear mixed-effects modeling software package (NONMEM) (Version V, level 1.1, GloboMax LLC, Hanover, USA) using pharmacokinetic data from 140 renal transplant recipients collected during the first six months after transplantation. Details about this model, designed to describe the pharmacokinetics of MPA, can be found elsewhere [16]. Briefly, for a patient with a creatinine clearance of 48 ml/min, albumin concentration of 30 g/l and a CsA daily dose of 500 mg, pharmacokinetic parameter values were: 4.1 $hours^{-1}$ for the oral absorption rate constant (Ka) with a lag time of 0.21 hours, 91 L for the central volume of distribution (V1), 33 L/h for MPA oral clearance (CL/F), 237 L for peripheral volume of distribution (V2) and 35 L/h for intercompartmental clearance. The estimated between-patient variability for Ka, V1, oral CL and V2 was 111%, 91%, 31% and 102% respectively. The variability within a patient in time could be estimated for Ka, V1 and oral CL and was 116%, 53% and 20%, respectively. The model partly explained variability between and within patients by establishing correlations between patient characteristics and oral CL: typically, oral CL decreased from 39 to 33 L/h when renal function, expressed as creatinine clearance, improved from 10 to 60 mL/min, oral CL decreased from 33 to 19 L/h when albumin concentrations increased from 30 to 50 g/L and oral CL decreased from 41 to 33 L/h when CsA daily dose was lowered from 1000 to 700 mg. Given these correlations, a decrease in oral CL was described by the model in the first months after transplantation as renal function recovers, albumin concentrations rise and CsA doses are tapered.

By the NONMEM model, MPA oral CL was estimated for every individual on every sampling occasion using Bayesian estimation [17]. A Bayesian estimate of a pharmacokinetic parameter is calculated

using concentration-time data from the concerned individual as well as information from the above described population pharmacokinetic model. With the Bayesian estimates for MPA oral CL, 2 dosing regimens were simulated: a fixed dose (FD) regimen and a concentration controlled (CC) regimen. This was done for 45 of the 140 renal transplant recipients, namely those from the intermediate target MPA AUC group of the RCCT. This selection was done because these patients had approximately the same starting dose and dose range in the RCCT as in the computer simulation, excluding a possible effect of MMF dose on MPA oral CL. For the FD-regimen the area under the plasma concentration-versus-time-curve (AUC) was calculated using the following equation 1:

MPA AUC [mg*h/L] = MPA dose / MPA CL [L/h] (Eq. 1)

In equation 1, MPA AUC is the MPA exposure, MPA oral CL is the Bayesian estimate, and MPA dose was 739 mg BID on every sampling occasion, which corresponds to the standard MMF dose of 1000 mg BID. Although doses are normally denoted as the quantity of administrated MMF, equation 1 uses the MPA dose because it concerns the calculation of exposure to MPA and of MPA oral clearance, not of MMF. MPA exposure was derived for each individual patient on each occasion.

For simulation of the CC-regimen the same estimates for MPA oral CL were used. In this simulation, MPA doses were adjusted based on the calculated MPA AUC of the preceding dose. Starting MPA dose was 739 mg BID (equalling a MMF dose of 1000 mg BID) for every patient and MPA AUC for the first sampling occasion (day 3 after transplantation) was calculated according to equation 1. Based on this AUC, the subsequent MPA dose was adjusted, targeting at an AUC level of 45 mg*h/L, which is the mean of the recommended therapeutic window of 30-60 mg*h/L [10]. The following equation was used, assuming linear pharmacokinetics:

Subsequent MPA dose [mg] = (45 / preceding MPA AUC [mg*h/L]) * preceding MPA dose [mg] (Eq. 2)

To obtain the (clinically applied) MMF dose that equals the subsequent MPA dose, MPA doses were divided by 0.739 and were corrected to units of 250 mg. The maximum MMF dose change and maximum daily MMF dose were set at 1000 mg and 2000 mg BID, respectively. These limits were arbitrarily set because it was considered unlikely that clinicians would be willing to make dose changes of more than 1000 mg or to prescribe doses higher than 2000 mg BID in case of low exposure. For the following sampling occasion, the accompanying Bayesian estimate for MPA oral CL and the adjusted MPA dose were used to calculate the AUC (equation 1). The result could give rise to a new dose adjustment (equation 2), and so on.

Statistics

Statistical tests and calculations were performed with the software package SPSS 10.1 for Windows (SPSS Inc. Chicago, IL) or Microsoft Excel 97 SR-2 (Microsoft Corporation, Redmond, WA). Patient characteristics and pharmacokinetic data are presented as means with standard deviations or as median and range. Between-patient variability on every separate sampling occasion was expressed as coefficient of variation (CV, %). The χ^2 test was used to test for categorical differences between the FD and the CC regimen. A p-value of 0.05 was considered statistically significant.

RESULTS

MPA exposure was simulated for 45 patients by using a fixed-dose (FD) as well as a concentration-controlled (CC) regimen of MMF. Patient characteristics are summarised in Table 1. Results of both simulations are presented in Table 2 and Figure 1. In the CC-group a total of 163 MMF dose changes occurred during assessment of 312 AUC values. The smallest calculated MMF dose was 500 mg BID and the largest was 4500 mg BID. However, since the maximum MMF dose was arbitrarily set to 2000 mg BID, the MMF dose was increased only to this maximum value. Based on equation 2, the largest calculated dose change was an increase of 3250 mg after obtaining an AUC value of 11 mg*h/L on day 3 after transplantation; in this patient the maximum dose change of 1000 mg was applied. After the first MPA AUC measurement on day 3, the median MMF dose in the CC-group was doubled to 2000 mg BID. Twenty-eight of the 45 renal transplant patients received this maximum dose on the second occasion. Median doses gradually decreased towards 1500 mg BID on day 84 and 1000 mg BID on day 140 after transplantation (table 2).

After the first dose adjustment (day 7 after transplantation), 76% of patients on the CC regimen had MPA exposure within the target AUC range of 30-60 mg*h/L (figure 1 and table 2). This percentage was significantly higher than in the FD group, where only 13% of the patients had a MPA AUC between 30 and 60 mg*h/L ($p<0.001$). In the FD arm of the study, target AUC was not reached for the majority of patients (60%) until day 84 after transplantation. For every sampling occasion, the CC regimen produced significantly more AUC values within the therapeutic range than the FD regimen (table 2).

Figure 1 Box-Whisker plot of the comparison between a fixed dose and a concentration-controlled MMF dosing regimen during the first 140 days after renal transplantation.
The box indicates the upper and lower quartiles and the central line represents the median. The whiskers represent the 2.5% and 97.5% values. Extremes are denoted by an "x" and are values that are more than three box lengths from the upper edge of the box. AUC = area under the concentration-time curve. FD = fixed dose MMF dosing regimen. CC = concentration-controlled MMF dosing regime with maximum dose change of 1000 mg and maximum MMF dose of 2000 mg. The dotted lines represent the recommended target AUC range of 30-60 mg*h/L.

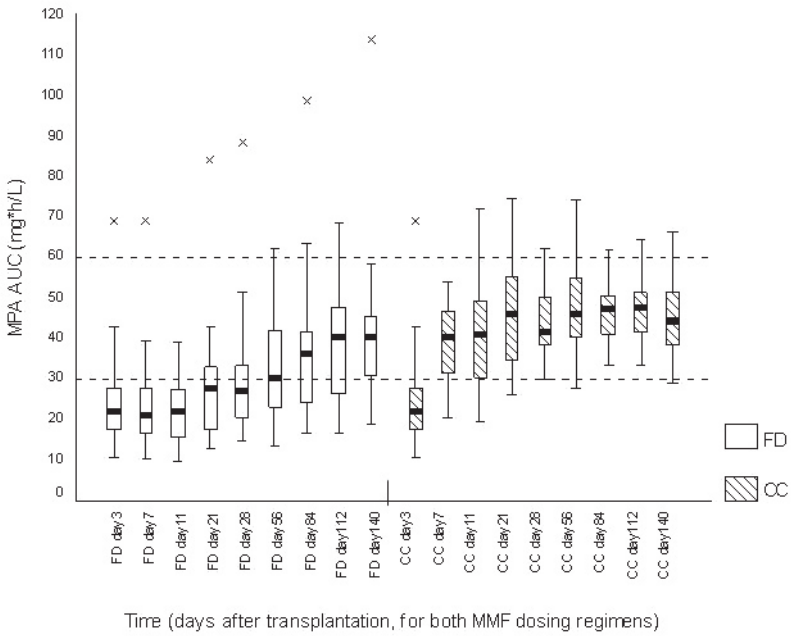

Table 1 Patient demographics.

Characteristic	**Mean±sd (range)**
Number of subjects	
Male	29
Female	16
Age (years)	47±13 (19-70)
Body weight (kg)	68±13 (37-96)
Delayed graft function	8
Creatinine clearance(mL/min)	
Day 3	29±19 (4-78)
Day 28	49±17 (7-97)
Day 140	57±16 (26-93)
Albumin concentration (g/L)	
Day 3	34±4 (23-45)
Day 28	37±6 (23-47)
Day 140	41±5 (32-48)
cyclosporine daily dose (mg)	
Day 3	583±184 (260-900)
Day 28	470±167 (240-975)
Day 140	327±150 (0-1000)

Data are presented as mean with standard deviation and range between parentheses. The indicated days are the days after renal transplantation.

Table 2 Comparison between the concentration-controlled and the fixed-dose MMF regimen.

Sample occasion	MPA CL (L/h)	Fixed dose n=45				Concentration-controlled n=45				p-value
		MMF dose (mg bid)	MPA AUC (mg*h/L)	BPV (%)	% on target	MMF dose (mg bid)	MPA AUC (mg*h/L)	BPV (%)	% on target	
3	36 (11-70)	1000	22 (11-69)	44	16	1000 (-)	22 (11-69)	44	16	
7	36 (11-72)	1000	21 (10-69)	42	13	2000 (750-2000)	41 (20-54)	24	76	<0.001
11	36 (12-75)	1000	22 (10-62)	43	16	2000 (750-2000)	41 (20-72)	28	74	<0.001
21	32 (9-57)	1000	28 (13-84)	47	34	2000 (750-2000)	46 (26-75)	28	71	0.001
28	29 (8-50)	1000	27 (15-88)	46	46	1750 (500-2000)	42 (30-62)	18	95	<0.001
56	25 (10-53)	1000	30 (14-77)	41	46	1750 (500-2000)	46 (28-83)	26	79	0.002
84	23 (7-44)	1000	36 (17-99)	43	60	1500 (500-2000)	47 (34-62)	16	91	0.002
112	22 (8-44)	1000	41 (17-95)	42	57	1250 (500-2000)	48 (33-75)	20	86	0.008
140	20 (7-39)	1000	41 (19-114)	44	67	1000 (500-2000)	44 (29-73)	21	91	0.016

Values for MPA oral CL, MPA AUC and MMF dose are expressed as median with minimum and maximum value between parentheses. Sample occasion corresponds to the days after transplantation. BPV, between-patient variability, defined as the CV (%) for the AUC calculation per sampling occasion. Target is defined as a MPA AUC between 30 and 60 mg*h/L. The concentration-controlled MMF dosing regime included a maximum dose change of 1000 mg and a maximum MMF dose of 2000 mg.

p-value for the comparison between the fixed-dose and the concentration-controlled MMF dosing regimen for the percentage of AUCs that are within the target range.

In the CC group, the CV for AUC estimates per occasion, as a measure for between-patient variability, was smaller than 30% on every sampling occasion following day 3 after transplantation, whereas in the FD group the CV was larger than 40% on every sampling occasion (table 2). Average CV values for all occasions were 44% in the FD group and 23% in the CC group. Furthermore, the extremely high AUC values (>85 mg*h/L) that were found in the FD group were not present in the CC regimen as can be seen from figure 1.

DISCUSSION

From a computer simulation model it is shown that TDM of MPA may result in more efficient dosing of MPA: in the concentration-controlled (CC) group more patients were within the recommended MPA AUC therapeutic window and less between-patient variability in exposure was observed (figure 1, table 2). From day 7 after transplantation, significantly more patients in the CC regimen reached target MPA exposure compared to the FD regimen. To accomplish this, large dose increases were necessary in the first week after renal transplantation with a doubling of the MMF dose from 1000 mg BID to 2000 mg BID in more than half of the patients. In the 3 so-called pivotal trials of MMF after kidney transplantation, it was concluded that there was no benefit of a daily MMF dose of 3 g compared to using 2 g daily, because with a 3 g dose an increased incidence of side effects without a further reduction in acute rejection was observed [1,18,19]. However, the present study suggests that for a subgroup of patients, with low MPA concentrations in the first weeks after transplantation and using MPA concentration monitoring, a temporarily increased dose may improve outcome. At 6 months median MMF dose in the CC-group will come down to 1000 mg BID.

This simulation was done before the start of a prospective randomised trial that will compare a concentration-controlled and a fixed-dose MMF regimen. Trial simulations are becoming increasingly important in order to select a clinical trial design that will generate the maximum amount of information on the drug under investigation [11-14]. For example, trial simulation guided the design of the RCCT trial, which contributed to successful execution and from which the data for the present simulation were extracted [14]. The present simulation demonstrates how quickly patients treated according to the CC-regimen will be on target, to what extent MMF dose changes will be necessary to achieve target, and the amount of variability in MPA AUC that will remain despite TDM.

With regard to between-patient variability, the CC regimen especially prevented the occurrence of extremely high (>85 mg*h/L) and low (<20 mg*h/L) AUC values (figure 1, table 2). Nevertheless, CV values for AUC estimates per sample occasion were still considerable in the CC group. This variability is mainly caused by variability within patients in time. Within-patient variability reduces the efficiency of TDM and may cause the need for frequent monitoring of MPA to keep AUC within the recommended range, especially in the first period after transplantation [10]. Besides, also the correction of the calculated MMF dose to units of 250 mg may have contributed to some extent to the variability of MPA AUC observed in the CC group.

Dose adjustments in this simulation were calculated based on estimates for MPA oral CL, which were derived by a population pharmacokinetic model. The model accounted for between- and within-patient variability for oral CL caused by renal function, albumin concentration, and CsA dose, thereby improving the accuracy of the estimates for MPA oral CL [16]. Furthermore, the model described the decrease in MPA oral CL in the first months after transplantation (table 2), that has also been observed in several pharmacokinetic studies [4,7]. Consequently, calculated median MMF doses decreased in the CC-group after the first three weeks after transplantation to keep AUC on target. The decrease in oral CL may be explained by a decrease in MPA free fraction as a result of improving renal function and rising albumin concentrations after transplantation [6,7,20]. Because it is believed that only the free fraction is available for elimination, a decrease in free fraction may cause a decrease in CL. The most pronounced changes in CL over time are observed in patients with delayed graft function [7]. Another explanation for the decrease in oral CL may be tapering of the CsA dose, which could decrease the suggested inhibitory effect of CsA on the enterohepatic recirculation of MPA [21].

In some cases application of equation 2 resulted in high MMF doses. However, the extent of dose increase and the maximum MMF dose were limited since rigorous interventions are not likely to be applied in daily clinical practice. Instead, doses could be increased to a maximum of 2000 mg and doses could not be changed with more than 1000 mg per occasion in order to simulate a MMF dosing regimen that best reflects clinical practice.

An important limitation during simulation of the CC regimen is that the clinical status of patients (e.g. side effects) was not taken into account when a dose change was applied, which may not reflect clinical reality. Also in the FD regimen, where a patient could have no other MMF dose than 1000 mg, the clinical situation of a patient was not taken into consideration. This study was designed to solely generate insight into the effect of TDM on MPA exposure without the possible influence of disturbing variables. Consequently, this simulation provides a prediction of the maximum beneficial effect that TDM can have on MPA exposure during pharmacotherapy. In clinical practice, TDM will ensure that more patients are on target more quickly, but it may take more time than predicted, and more between-patient variability in MPA AUC may occur in the CC regimen.

The population from which data in this study were simulated used CsA as concomitant immunosuppressive therapy. As mentioned above, CsA is believed to decrease the exposure to MPA in renal transplant recipients, probably through interruption of the enterohepatic recirculation of MPA [8,9,21]. Consequently, the results of this analysis are not directly applicable to renal transplant recipients that use MMF in combination with tacrolimus. TDM may have the same impact on MPA exposure in tacrolimus-treated patients, as seen in this simulation, but the extent and frequency of MMF dose adjustments may be less intensive, especially in the first weeks after transplantation.

CONCLUSION

By application of TDM of MPA it will be possible to individualise MMF therapy in renal transplant patients that use CsA as concomitant immunosuppressive therapy. Between-patient variability is reduced and target AUC is reached more rapidly when compared to a fixed-dose MMF dosing regimen. Perhaps most importantly, the very high (>85 mg*h/L) and the very low (<20 mg*h/L) AUC values are avoided in the CC group. Furthermore, it appears that larger MMF doses as currently recommended are necessary shortly after transplantation to reach target AUC levels as soon as possible. Although current data suggest that MPA AUC values between 30 and 60 mg*h/L are associated with less acute rejection episodes and fewer side effects [22], it can not be concluded yet that TDM improves clinical outcome with MMF therapy. The results of this computer simulation, performed with data from extensively monitored actual patients, should be interpreted as a rationale for the performance and as a prediction of the outcome of prospective trials comparing a CC with a FD dosing regimen. Such studies are currently ongoing.

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Chapter 3.2

Within-Patient Variability of Mycophenolic Acid Exposure

Therapeutic Drug Monitoring From a Clinical Point of View

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ABSTRACT

Exposure to mycophenolic acid (MPA) is highly variable between patients on standard dose mycophenolate mofetil (MMF) therapy. In addition, MPA exposure increases over time posttransplant and exposure is predictive for the development of acute rejection. Consequently, therapeutic drug monitoring (TDM) of MPA may improve clinical outcome, although a large within-patient variability could be a limitation. This study was designed to analyze the extent of within-patient variability of MPA exposure for area-under-the-curve (AUC_{0-12}) and pre-dose concentrations (C_0). For 9 occasions during the first 5 months after transplantation, AUC_{0-12} and C_0 values from 45 renal transplant recipients, all using cyclosporine and corticosteroids, were divided into quartiles. When AUC_{0-12} or C_0 changed 1, 2 or 3 quartiles within a patient from one occasion to the next, a score of respectively 1, 2 or 3 points was assigned. Doing this for all 8 between occasion intervals, the maximal score for within-patient variability could be 8 x 3 = 24 per patient. For AUC_{0-12}, the median overall score was 3.4 of maximal 24. For C_0 measurements this score was significantly higher: 6.0 ($p<0.001$). The higher overall score for C_0 was explained by more quartile changes during the first weeks after transplantation. It is concluded that within-patient variability for MPA exposure is low in kidney transplant recipients during the first 5 months after transplantation. In the first weeks after transplantation, within-patient variability is larger for C_0 than for AUC_{0-12}.

INTRODUCTION

Mycophenolate mofetil (MMF) is used for the prevention of acute graft rejection after solid- organ transplantation. It is a widely used immunosuppressive drug as 79% of renal transplant recipients, 52% of pulmonal transplant recipients and 75% of cardiac transplant recipients use MMF at discharge from the hospital [1]. MMF was introduced to the market with a dose recommendation for adults of 1 g twice daily, because the 3 so-called pivotal trials of MMF after kidney transplantation showed no further reduction in acute rejection rates with 1.5 g twice daily, whereas an increased incidence of side effects was observed [2]. Since the approval of MMF by the FDA in 1995, MMF has been regarded as "simple to use without monitoring" [3]. However, accumulating evidence from pharmacokinetic studies demonstrates a 10-fold between-patient variability in the exposure to mycophenolic acid (MPA), the active compound of MMF, in solid organ transplant recipients on standard dose therapy [4]. Besides, MPA exposure increases over time after transplantation with a fixed MMF dose, co-medication like cyclosporine (CsA) influences exposure to MPA and, importantly, correlations between MPA exposure and risk for acute rejection have been established [5,6,7].

Based on this pharmacokinetic evidence, under- or overimmunosuppression may be caused in a proportion of patients when MMF is used with the recommended fixed dose of 1 g twice daily. Consequently, monitoring of MPA exposure with subsequent MMF dose adjustment is receiving more and more attention [4,8]. Therapeutic drug monitoring (TDM) reduces between-patient variability in exposure to MPA and targets exposure, expressed as area-under-the-curve (AUC), to the currently adopted therapeutic window of 30-60 mg*h/L [9]. However, large within-patient variability could reduce the efficiency of TDM as variations over time cannot be controlled and may drive exposure away from the therapeutic window. This may cause the need for more frequent monitoring. Several studies assessed the extent of within-patient variability and found that it was large [10-12], but not all of these studies separated the variability caused by the increasing exposure over time from the overall within-patient variability. Also, from these studies the link between variability and need for dose adjustments could not be made. This study was designed to quantify within-patient variability of MPA exposure

from a clinically relevant perspective, both for AUC and pre-dose levels (C_0). Given a 10-fold between-patient variability, with a range of MPA AUC values from 10 to 100 mg*h/L, and an established target range from 30 to 60 mg*h/L, for a clinician it is not extremely relevant if the AUC is 36 or 50 mg*h/L. What clinicians would want to avoid is gross over- or underimmunosuppression, i.e. increasing dose if patients are below target, or decreasing dose in those above target. The results from this knowledge can contribute to determine how often MPA exposure should be measured for optimal exposure and which exposure parameter would be the most suitable one.

PATIENTS AND METHODS

Patients and sampling procedure

Concentration-time data from 45 renal transplant recipients who participated in a Randomised Concentration Controlled Trial (RCCT) were analysed retrospectively. A detailed description of the methods and results of the RCCT can be found elsewhere [5,6]. Briefly, the RCCT investigated the relationship between MPA exposure (AUC, C_0 and maximum concentration) and outcome (risk for acute rejection and side effects). Adult patients were randomly assigned to three target MPA AUC groups: low, intermediate or high. All patients received CsA and corticosteroids as concomitant immunosuppressive therapy. For the present study only the data from the intermediate target AUC group (target AUC: 32.2 mg*h/L, starting MMF dose 950 mg twice daily) were analysed because this MMF dose best reflects current clinical practice.

Serial concentration-time samples were collected on 9 occasions during the first 5 months after transplantation: days 3, 7, 11, 21 and months 1, 2, 3, 4 and 5. Plasma samples for description of full 12-h concentration-time profiles on the first three sampling occasions were taken predose, 0.33, 0.66, 1.25, 2, 6, 8 and 12 hours after oral intake of MMF. For the 6 remaining occasions, serial plasma samples were taken according to a limited sampling strategy predose, 0.33, 0.66, 1.25, and 2 hours postdose. The concentrations at 6, 8 and 12 hours were estimated by an empiric equation [6]. Samples were analysed using a validated HPLC method [13]. $AUC_{0\text{-}12}$ was estimated with the linear trapezoidal rule.

Assessment of within-patient variability

$AUC_{0\text{-}12}$ and C_0 values, normalised to 1 g of MMF, for every separate occasion were divided into quartiles. For every patient the time-course of both $AUC_{0\text{-}12}$ and C_0 was followed over the 9 sampling occasions, determining in which quartile exposure fell per occasion. Within-patient variability was quantified by calculating a score that depended on the extent the MPA exposure changed quartiles. When $AUC_{0\text{-}12}$ or C_0 changed 1, 2 or 3 quartiles within a patient from one occasion to the next, a score of respectively 1, 2 or 3 points was assigned. When exposure did not change quartiles, a score of 0 was applied. For all 8 between occasion intervals, a maximal possible score for within-patient variability of 8 x 3 = 24 per patient could be assigned. This method for the analysis of within-patient variability roughly measures scaled variations within a patient over time, making it possible to discriminate between variations that most likely warrant a dose adjustment, namely those with a score of 2 or 3 points, and those that do not (score of 0 or 1).

Statistics

Statistical tests and calculations were performed with the software package SPSS 10.1 for Windows (SPSS Inc. Chicago, IL) or Microsoft Excel 97 SR-2 (Microsoft Corporation, Redmond, WA). Data are presented as median with range unless otherwise stated. The Wilcoxon signed rank test was used to test for statistical differences between AUC_{0-12} and C_0. P = 0.05 was considered statistically significant.

RESULTS

For 45 renal transplant recipients (demographics are presented in table 1), the course of MPA exposure (AUC_{0-12} and C_0) was analysed over the 9 sampling occasions, assessing within-patient variability by analysing when exposure changed quartiles. The course of dose normalised MPA AUC_{0-12} and MPA C_0 for every patient is shown in figure 1 and 2 respectively. Both figures indicate that most patients exhibit quite stable exposure during the course of the study, although several patients occasionally show extreme variations. Besides the within-patient variability, these figures also visualise the between-patient variability. For example, on day 28 after transplantation the MPA AUC_{0-12} ranged from 13.8 mg*h/L to 94.7 mg*h/L, showing an almost 10-fold variability.

The quartile cut-off values for days 3 and 28 and month 5 are presented in table 2. During the study period the cut-off values increase, reflecting the well known increase of MPA exposure with time after transplantation, which is also apparent in figures 1 and 2.

Of all changes of exposure between 2 successive occasions for AUC_{0-12}, 36% had a score of 1 point, 6% had a score of 2 points and 1% had a score of 3 points. For C_0 these values were respectively 42%, 16% and 1%. The results for the within-patient variability for both MPA AUC_{0-12} and MPA C_0 are summarised in table 3. The median score for within-patient variability of AUC_{0-12} is statistically significantly smaller than for C_0: 3.4 versus 6.0 of maximum 24 respectively ($p<0.001$). The higher overall score for C_0 is a result of more within-patient variability during the first weeks after transplantation considering the significantly higher score for C_0 on days 7 through 56 after transplantation. During this period, the median score for AUC_{0-12} is always 0 (mean score is always less than 0.6 of maximum 3), while the median score for C_0 is always 1 (mean score is always higher than 0.65).

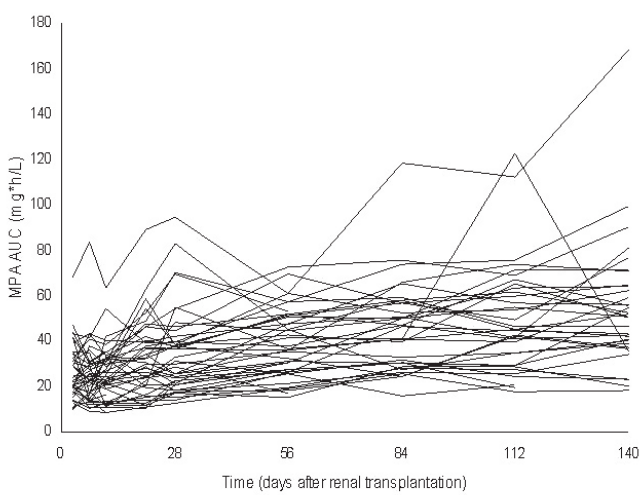

Figure 1 The course of dose normalised MPA AUC_{0-12} for every patient during the first 5 months after renal transplantation.

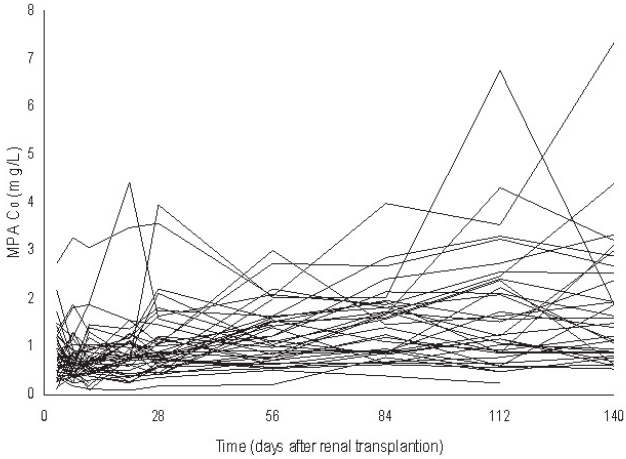

Figure 2 The course of dose normalised MPA predose concentration (C_0) for every patient during the first 5 months after renal transplantation.

Table 1 Patient demographics. Data are presented as mean with standard deviation and range.

Characteristic	**Mean±sd (range)**
Number of subjects	
Male	29
Female	16
Age (yr)	47±13 (19-70)
Body weight (kg)	68±13 (37-96)
Delayed graft function	8
Creatinine clearance(mL/min)	
Day 3	29±19 (4-78)
Day 28	49±17 (7-97)
Day 140	57±16 (26-93)
Albumin concentration (g/L)	
Day 3	34±4 (23-45)
Day 28	37±6 (23-47)
Day 140	41±5 (32-48)
Cyclosporine daily dose (mg)	
Day 3	583±184 (260-900)
Day 28	470±167 (240-975)
Day 140	327±150 (0-1000)

Table 2 Quartile cutoff values.

Day after transplantation	Quartile	Cutoff values for MPA AUC_{0-12} (mg*h/L)	Cutoff values for MPA C_0 (mg/L)
3	25	17.9	0.45
	50	21.9	0.74
	75	32.3	1.13
28	25	22.0	0.62
	50	32.8	0.82
	75	40.9	1.20
140	25	38.1	0.87
	50	50.9	1.49
	75	64.1	2.60

Table 3 Scores for within-patient variability for AUC_{0-12} and C_0.

Between occasion interval (days)	Maximum score	Within-patient variability AUC_{0-12}		Within-patient variability C_0		p-value
		Median	Mean	Median	Mean	
3-7	3	0	0.58	1	0.91	0.06
7-11	3	0	0.49	1	0.95	0.007
11-21	3	0	0.51	1	0.76	0.05
21-28	3	0	0.41	1	0.66	0.05
28-56	3	0	0.45	1	1.00	0.006
56-84	3	0	0.44	1	0.62	0.08
84-112	3	1	0.57	1	0.60	0.8
112-140	3	0	0.55	0	0.55	1.00
Overall score (range)	24	3.4 (0-14)	4.1	6.0 (0-11)	6.3	<0.001

Between occasion interval concerns days after transplantation. Both median and mean scores are presented for both AUC_{0-12} and C_0.
P-value for the difference in score for within-patient variability between AUC_{0-12} and C_0.

DISCUSSION

In recent literature on MMF a lot of attention has been given to the potential contribution of TDM for MPA [4,9,14]. MMF dose adjustments based on MPA plasma concentrations reduce between-patient variability, targeting more patients to the currently recommended therapeutic AUC window of 30-60 mg*h/L. However, whether TDM also leads to improved efficacy and less toxicity has still to be proven. This is currently under investigation in three randomised trials. Meanwhile, large within-patient variability of MPA exposure could be an important limitation for efficient and effective TDM. This study reveals that the extent of within-patient variability for MPA AUC_{0-12}, measured as a score for changes of quartiles from one sampling occasion to the next, is low. The extent of within-patient variability for AUC_{0-12} does not differ to a clinically relevant extent between the separate occasion intervals (table 3), indicating that variability shortly after transplantation is not different from the variability during later

time periods, with the exception of greater within-patient variability for C_0 than for AUC_{0-12} in the first weeks after transplantation. The most important implication of these results is that when a patient has a certain MPA exposure resulting from a certain MMF dose, the exposure is expected to be stable, apart from the structural increase of exposure over time. Some (random) changes may occur, but these are in most cases small (93% of scores for within-patient variability for AUC_{0-12} and 83% for C_0 are 0 or 1) and are unlikely to cause over- or underimmunosuppression that warrant a dose adjustment. The argument that TDM for MMF would not be necessary at all is contradicted by the fact that variability of MPA exposure between patients was large (e.g. 10-fold variability on day 28 posttransplant) and that in the present cyclosporine treated study population, at first AUC_{0-12} measurement (day 3 after transplantation), 71% of patients had a MPA exposure outside of the 30 to 60 mg*h/L target window.

Given the low within-patient variability for C_0, dosing based on pre-dose levels may be a feasible option as this is more practical than dosing based on full AUC. However, the significantly higher score for within-patient variability in the first weeks after transplantation may pose a drawback. Moreover, within-patient variability may be higher as estimated in this analysis when one considers the fact that patients participating in the RCCT were extensively instructed to take their medication at the correct time. In routinely monitored patients, the timing of medication intake may be more randomly distributed, causing more variability. Another problem with the use of C_0 as measure for MPA exposure is the weak correlation between predose levels and AUC_{0-12} (r^2=0.69 in this study), which suggests that C_0 does not always reflect exposure adequately. These aspects make C_0 less attractive to guide MMF dose individualisation.

The applied method for quantification of within-patient variability was used to detect variations within a patient over time that are clinically relevant and may warrant a dose adjustment. Subtle variations within a patient, which formally contribute to variability but are thought to lack clinical relevance, are ignored (score = 0). An advantage of this method is that it corrects for the structural increase of MPA exposure over time posttransplant, which is shown by the increase of the quartile cut-off values (table 2). Only the random variability is measured, not the structural changes over time. This also causes a disadvantage of the method: a subject with an almost equal MPA exposure on two successive occasions could change from one quartile to another (score = 1), causing an overestimation. Another disadvantage of the method is that large exposure changes within a patient within the upper quartile, occasionally did not contribute to within-patient variability causing an underestimation. Nevertheless, the applied method is suitable to provide an indication about the magnitude of within-patient variability and about the risk that this variability could cause over- or underimmunosuppression that warrant dose adjustments to get exposure on target.

CONCLUSION

Within-patient variability in MPA exposure is low, not only during the overall study period, but also during separate occasion intervals. Based on this observation the following TDM scheme may prove to be useful: a measurement of MPA exposure, preferably by (abbreviated) AUC, in the first week after transplantation to determine the optimal MMF dose, thereby reducing between-patient variability and targeting exposure to the therapeutic window. A second measurement is probably useful 1 or 2 months after transplantation to compensate for the increase of MPA exposure over time. Because of the low within-patient variability, significant changes in MPA exposure are unlikely making more frequent monitoring unnecessary, unless major changes in patient condition or co-medication occur.

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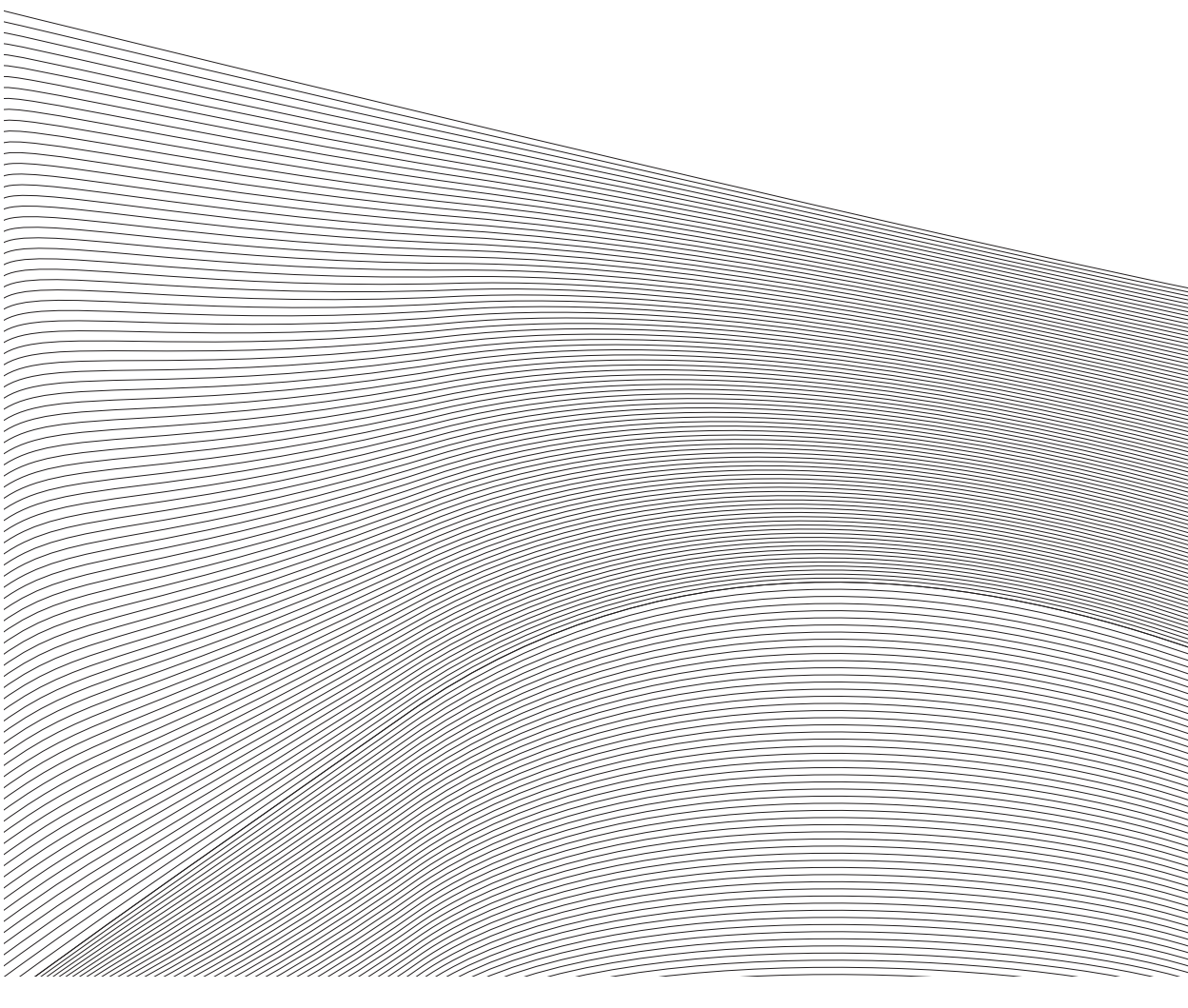

Chapter 4

PHARMACOKINETICS OF MYCOPHENOLATE MOFETIL IN STEM CELL TRANSPLANT RECIPIENTS

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ABSTRACT

Mycophenolate mofetil (MMF), a prodrug of mycophenolic acid (MPA), is increasingly used in the prophylaxis of graft-versus-host disease (GVHD) after haematopoietic stem cell transplantation (HCT). Few pharmacokinetic data are available about the use of MMF for this indication. This case series aimed at analysing the pharmacokinetics of MMF in a population of HCT recipients representative for every day practice.

From fifteen HCT recipients, serial plasma samples were taken after twice daily oral intake of MMF. Plasma concentrations of total MPA and its glucuronide metabolites, as well as free MPA were quantified.

Median apparent oral MPA clearance (CL), half-life, and total MPA AUC_{0-12} (normalised to 1000 mg MMF) were respectively 56 L/h, 2.3 hours, and 18.0 mg*h/L. Total MPA concentrations were below 2 mg/L 8 hours after MMF administration, indicating reduced enterohepatic recirculation. Median free MPA AUC_{0-12} (normalised to 1000 mg MMF) was 224 μg*h/L. MPA CL significantly increased with the number of co-administered highly albumin bound drugs (p=0.05).

Due to high apparent oral CL, total MPA exposure in HCT recipients is low and half-life is short compared to reference values from renal transplantation. Exposure may be improved in HCT recipients by higher or more frequent MMF dosing.

INTRODUCTION

Mycophenolate mofetil (MMF) is a prodrug of mycophenolic acid (MPA), and is used as an immunosuppressive drug to prevent acute rejection following solid organ transplantation (1,2). The pharmacokinetics of MPA are described by a rapid absorption from the gut with maximum MPA peak concentrations generally occurring within 1 hour after oral MMF administration (3). MPA is primarily metabolised in the liver by uridine diphosphate glucuronosyl transferase to form the metabolites phenolic MPA glucuronide (MPAG) and to a lesser extent acyl MPA glucuronide (AcMPAG) (4). The latter is pharmacologically active, and has been linked to the occurrence of MMF related adverse effects (5,6). The glucuronide metabolites are excreted into bile, prompting an enterohepatic recirculation (EHC), which causes a second MPA peak concentration approximately 6 to 12 hours after administration. Finally, the glucuronide metabolites are excreted by the kidneys (3). MPA is highly bound (98%) to albumin, and has been shown to be displaced *in vitro* by high concentrations of MPAG, which has an albumin binding of 82% (3), and which accumulate during renal impairment (7).

MMF has been shown to be a potent immunosuppressive drug with beneficial effects on acute rejection rates and on long-term outcomes compared to azathioprine (8,9). The success of MMF within solid organ transplantation has triggered the increasing application of MMF in the prophylaxis and treatment of acute and chronic graft-versus-host disease (GVHD) after haematopoietic stem cell transplantation (HCT) as well as to promote engraftment in nonmyeloablative HCT (10-12). MMF is used for this indication to further reduce the incidence of acute GVHD as an alternative for methotrexate, and is supposed to have a more favorable toxicity profile, especially with regard to the incidence of mucositis (10,11,13-15). MMF dose and dose interval applied in HCT recipients are largely based on pharmacokinetic data from renal transplant studies, as such data are scarce in HCT. This means that the starting MMF dose generally is 15 mg/kg twice daily p.o., which in most HCT patients results in the standard MMF dose recommended in renal transplantation of 1000 mg twice daily. Preliminary pharmacokinetic data from HCT recipients after standard MMF dosing showed low MPA trough levels

compared to data from renal transplantation (10,16-18). Confirmation of low MPA exposure, assessed by pre-dose levels and area-under-the-curve (AUC), was provided by three studies in HCT patients treated with nonmyeloablative conditioning (12,19,20). Standard twice daily oral MMF dosing also seemed to result in low MPA exposure in four HCT patients after myeloablative conditioning (21). These results suggest that in HCT the pharmacokinetics of MMF are different compared with renal transplantation. This pilot study aimed at describing the pharmacokinetics of MPA, MPAG, AcMPAG and free MPA in a case series of allogeneic HCT recipients representative for every day practice after application of a MMF dosing regimen based on renal transplant data.

PATIENTS AND METHODS

Patients and treatment

Patients included in this case series received an allogeneic peripheral stem cell transplant between May and November 2004 at the Erasmus University Medical Center-Daniel den Hoed Cancer Center. This was a pilot study, with the aim to get a representative impression of the pharmacokinetics of MMF in an unselected cohort of allogeneic HCT recipients. Patients were included regardless of underlying disease, conditioning regimen, donor type or patient condition. Patients were included after they gave informed consent.

The day of stem cell transplant is designated day 0. Patients up to 40 years of age with a related donor received a standard myeloablative conditioning regimen consisting of cyclophosphamide 60 mg/kg i.v. on days –5 and –4 and total body irradiation (TBI) 6 Gy on days –2 and –1. A nonmyeloablative conditioning regimen was applied in patients older than 40 years. This regimen consisted of fludarabine 30 mg/m^2 i.v. on days –5 to –3 and TBI 2 Gy on day –1. Patients who received stem cells from a matched unrelated donor received the same conditioning regimens as patients with a related donor. In addition, they were given rabbit antithymocyteglobuline (ATG) 2 mg/kg i.v. on days –7 to –4. For prevention of serum sickness, prednisone was administered in patients receiving ATG in doses of 0.5 mg/kg i.v. twice daily on days –7 to –4 and once daily on days –3 to +7.

Immunosuppressive therapy for prophylaxis of GVHD consisted of cyclosporine (CsA) and MMF. CsA was started on day –3. In patients receiving myeloablative conditioning, intravenous CsA (Sandimmune®) was administered in doses of 1.5 mg/kg twice daily. When these patients could tolerate oral medication, they were converted to oral CsA in an identical dose (Neoral®). In patients receiving nonmyeloablative conditioning, oral CsA (Neoral®) was given twice daily in doses of 6.25 mg/kg. CsA trough levels, determined in whole blood using the EMIT assay, were targeted at 250-350 mg/L, but higher levels were accepted during the first week in patients treated with nonmyeloablative conditioning. MMF was started 5 to 10 hours after transplantation with a dose of 15 mg/kg orally twice daily, rounded up or down to the nearest 250 mg MMF increment. Thereafter, twice daily MMF doses were adjusted to keep total MPA trough levels > 1 mg/L. This target trough level was based on knowledge from renal transplantation, where a therapeutic window for total MPA trough levels has been adopted of 1.0 to 3.5 mg/L (22).

Infection prophylaxis consisted of ciprofloxacin 500 mg orally twice daily and fluconazole 200 mg orally once daily starting at the first day of the conditioning regimen, until the granulocyte count was above 0.5x10^9/L. All patients received valaciclovir 500 mg three times daily starting at the first day of the conditioning regimen until one year posttransplant, except patients at risk for cytomegalovirus (CMV), who were given valganciclovir 450 mg once daily. Patients at risk for CMV were defined as patients

who were CMV-IgG positive or had a CMV-IgG positive donor and used more than 40 mg prednisolone per day after day 7, or patients with a CMV-PCR above 500 replicates. (CMV-PCR was routinely determined every week). Every patient received co-trimoxazole 480 mg once daily for prevention of *Pneumocystis Carinii* infection when ciprofloxacin and fluconazole were stopped, until one year after transplantation.

Sampling procedure

Serial concentration-time samples (7 mL per sample) for analysis of the pharmacokinetics of MMF were collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes before and 0.5, 1, 2, 4, 6, 8 and 12 hours after oral dosing. In some patients, the time points of sample drawing were different due to protocol violations, but these concentration-time profiles were still suitable for pharmacokinetic analysis. Patients were not required to fast. After collection, EDTA blood samples were immediately centrifuged at 3000 *g*, and the plasma was stored frozen at –80 °C until analysis.

Bioanalytic analysis

Quantification of MPA and MPAG concentrations in the plasma samples was simultaneously done with high-performance liquid chromatography (HPLC) according to the method described by Shipkova (23) with several modifications. 25 µL internal standard solution (tolmetine 120 mg/L in methanol) and 75 µL of acetonitrile was added to a 100 µL plasma sample in a 1.5 mL polypropylene tube and was vortex-mixed for 10 seconds. The sample was then centrifuged for 15 minutes at 32000 g. The supernatant was placed in a new polypropylene tube and centrifuged for 10 minutes at 32000 g. 20 µL of the resulting supernatant was used for injection into the chromatographic system. The HPLC system consisted of an autosampler (Midas, Spark Holland, Emmen, The Netherlands), a chromatographic pump (P100, Thermo Finnigan, Breda, The Netherlands), a diode array detector (UV6000, Thermo Finnigan, Breda, The Netherlands), a column oven (Varian ProStar, Middelburg, The Netherlands) maintained at 30°C, a 7.5 mm x 4.6 mm Platinum pre-column (Alltech, Breda, The Netherlands) and a 150 mm x 4.6 mm Platinum C_{18} reversed phase column (Alltech, Breda, The Netherlands). The mobile phase consisted of 25% acetonitrile and 75% 50 mmol/L phosphate buffer (pH 3.0) and was kept at a flow rate of 1.3 mL/min. MPA, MPAG and the internal standard were detected at 215 nm. Concentrations were calculated based on a four-point calibration curve determined with every separate run and with the peak area ratio of analyte and internal standard. The assay was validated for determination of MPA and MPAG in human plasma according to FDA guidelines (24) and based on internally prepared control samples with six different concentrations of MPA and MPAG. Control and calibration samples were frozen at –20 °C before they were used. The limit of quantification was 0.063 mg/L for MPA and 1.31 mg/L for MPAG. The assay was found to be linear to 45 mg/L for MPA and to 150 mg/L for MPAG (correlation coefficient >0.99). The within-days coefficient of variation (CV) ranged from 2.0% to 2.4% for MPA and from 2.2% to 8.5% for MPAG. The between-days CV ranged from 2.0% to 6.7% for MPA and from 6.7% to 11.0% for MPAG. The accuracy of the assay, defined as the percentage of recovery of MPA and MPAG from the control samples was for MPA in the range of 92-103% and for MPAG in the range of 95-108%.

Free MPA and AcMPAG concentrations were quantified in the laboratory in Goettingen, Germany. Free MPA was determined with a liquid chromatography-tandem mass spectrometry method described elsewhere (25). This method needed 300 µL plasma, the limit of quantification for free MPA was 0.5 µg/L and the method was linear to 1000 µg/L. Between-days CV was <10%. Determination of the AcMPAG metabolite was done with a HPLC method described elsewhere (26). This method needed 200 µL plasma, the limit of quantification for AcMPAG was 0.1 mg/L and the method was linear to 10 mg/L. Between-days CV was ≤14%.

Pharmacokinetic analysis

The concentration-time data were analysed using WinNonlin version 4.1 (Pharsight Corporation, Mountain View, Calif., USA). A noncompartmental model with extravascular input for plasma data was used to obtain estimates for total MPA, MPAG, AcMPAG and free MPA, maximum concentration (C_{max}), time to maximum concentration (T_{max}), volume of distribution (V) and AUC. Because steady-state conditions could be assumed in all patients, AUC_{0-12} values were estimated using the logarithmic trapezoidal rule. AUC_{0-12} values were dose normalised to 1000 mg MMF to facilitate comparison with the literature. MPA clearance was calculated by dividing the MMF dose by total MPA AUC_{0-12}. Since bioavailability (F) could not be quantified (intravenous data were not available), CL values correspond to the apparent oral values of this parameters which is the ratio of CL/F. MPA free fraction was calculated by dividing free MPA AUC_{0-12} by total MPA AUC_{0-12} times 100%. This way of calculation gives the average free fraction during a MMF dosing interval, which was a valid method as the free fraction was constant over the range of observed total MPA concentrations (plot not shown). In addition, the free fraction was constant over a dosing interval (data not shown). Half-life ($t_{½}$) of MPA was calculated based on the estimates for CL and V (27).

Statistics

Statistical tests were performed with the software package SPSS 10.1 for Windows (SPSS Inc. Chicago, IL, USA). Pharmacokinetic data and patient characteristics are presented as median and range as data were not normally distributed as tested with the Shapiro-Wilk test. Spearman's test for correlation (r_s) and Wilcoxon rank sum test were used for statistical testing. A p-value of 0.05 was considered statistically significant.

RESULTS

Patients

Full concentration-time profiles of MMF were drawn from 16 HCT recipients. One of these 16 patients is presented separately as a case report because of extreme patient demographics (serum albumin level of 15 g/L) and results (see case report) (28). Patient characteristics are summarised in table 1. Ten patients received their transplant from a related donor. Eleven patients recieved a myeloablative conditioning regimen (table 1). One of these patients, younger than 40 years of age and with a related donor, was treated with an alternative conditioning regimen, consisting of busulfan 1 mg/kg orally four times a day from day –9 to –6 and cyclophosphamide 1550 mg/m^2 i.v. from day –5 to –2, because this patient was not eligible to receive radiotherapy.

All patients received MMF through the oral route. Starting MMF doses ranged from 500 to 1500 mg twice daily with a median of 1000 mg twice daily. Median MMF dose at the time of pharmacokinetic sample drawing was 1500 mg twice daily (range: 750-2000 mg twice daily), indicating that dose increases were necessary in most patients to obtain a total MPA trough level > 1 mg/L. Median time after HCT of pharmacokinetic assessment was 8 days (range: 4-26 days). From 1 patient, 2 concentration-time profiles were taken on day 7 and 15 after HCT. In the pharmacokinetic analysis, these profiles were treated as if they originated from two different patients. This is unlikely to have had a large influence on the results, considering the descriptive aim of the present pilot study.

Table 1 Patient characteristics at the time of pharmacokinetic assessment.

	Median (range)
Female/Male (No.)*	6/9
Age (years)	32 (17-58)
Diagnosis (No.)	
AML/MDS-RAEBt	8
ALL	3
CML	1
NHL	2
Myeloproliferative syndrome	1
Conditioning regimen	
Myeloablative:	
Cyclosphosphamide+TBI	6
Cyclosphosphamide+TBI+ATG	4
Cyclophosphamide+busulfan	1
Nonmyeloablative:	
Fludarabine+TBI	3
Fludarabine+TBI+ATG	1
Neutropenia (neutrophil count < 0.5x109/L)	12
Body weight (kg)	70 (45-89)
Creatinine clearance (mL/min)	132 (46-265)
Albumin concentration (g/L)	33 (26-37)
MMF daily dose (mg) at time of pharmacokinetic assessment*	3000 (1500-4000)
Cyclosporine pre-dose level (ng/mL) at time of pharmacokinetic assessment	356 (198-562)

*Mycophenolate mofetil was always given twice daily.

In total, concentration-time curves were drawn from 15 haematopoietic stem cell transplant patients (excluding the patient presented as a case report). From one patient (male with AML) two concentration-time curves were drawn.

AML = acute myeloid leukaemia, MDS-RAEBt = myelodysplastic syndrome-refractory anaemia with excess of blasts in transformation, CML = chronic myeloid leukaemia, ALL = acute lymphoblastic leukaemia, NHL = non-Hodgkin's lymphoma, ATG = antithymocyte globulin, MMF = mycophenolate mofetil, TBI = total body irradiation.

At the time of pharmacokinetic assessment, acute GVHD was reported in 6 patients: 5 patients had grade I skin acute GVHD, for which they received triamcinolon ointment 0.1% topically twice daily. 1 patient had grade 3 acute GVHD which was treated with i.v. prednisolon 90 mg twice daily. 1 patient experienced graft rejection, while the remaining fourteen patients all reached complete donorchimerism within 3 months after transplantation. Reported side effects were mainly mucositis, present in 9 patients, and infections, which were present in 7 patients.

MMF Pharmacokinetics

The results of the pharmacokinetic analysis for total MPA, MPAG, AcMPAG and free MPA are presented in table 2 and figures 1 to 3. Figure 1 represents the concentration-time curves of every patient and shows that total MPA is rapidly absorbed, reaching peak concentrations within 2 hours after oral administration. The patient with the lowest maximum concentration of 2.6 mg/L received the lowest MMF dose of 750 mg twice daily and experienced mucositis at the time of sampling drawing. There was no clear explanation for the highest observed maximum total MPA concentration of 23 mg/L. Total MPA concentrations were below 2 mg/L for all individuals at 8 hours after dosing. In most profiles, there was no evidence of a second peak reflecting EHC. Also the individual concentration-time curves of free MPA (figure 2) and the average curves of total MPA, MPAG and AcMPAG, normalised to 1000 mg MMF (figure 3), show a minor contribution of EHC to drug exposure.

Table 2 Pharmacokinetics of MPA, MPAG, AcMPAG and free MPA in stem cell transplant recipients

Component	Parameter	Median (range) (n=16*)
Total MPA		
	AUC_{0-12} (mg*h/L)	25 (7.6-35)
	Dose normalised AUC_{0-12} (mg*h/L)	18 (10-35)
	CL (L/h)	56 (29-98)
	V (L)	184 (74-363)
	$t_{½}$ (h)	2.3 (0.8-5.7)
	C_{max} (mg/L)	8.0 (2.6-23)
	T_{max} (h)	1 (0.5-2.0)
	Trough concentration (mg/L)	0.63 (<LOQ-4.0)
MPAG		
	AUC_{0-12} (mg*h/L)	643 (219-1859)
	Dose normalised AUC_{0-12} (mg*h/L)	430 (146-1638)
	C_{max} (mg/L)	88 (30-260)
	T_{max} (h)	2.5 (1.0-6.0)
AcMPAG		
	AUC_{0-12} (mg*h/L)	3.5 (0.72-8.5)
	Dose normalised AUC_{0-12} (mg*h/L)	3.2 (0.48-8.3)
	C_{max} (mg/L)	0.66 (0.21-1.5)
	T_{max} (h)	2.0 (1.0-8.0)
Free MPA		
	AUC_{0-12} (µg*h/L)	275 (62-616)
	Dose normalised AUC_{0-12} (µg*h/L)	224 (56-411)
	C_{max} (µg/L)	76 (23-247)
	T_{max} (h)	1.0 (0.5-2.0)
	Trough concentration (µg/L)	5.2 (<LOQ-36)
Free fraction (%)		0.96 (0.54-3.1)

*In total, concentration-time curves were drawn from 15 haematopoietic stem cell transplant patients (excluding the patient presented as a case report). From one patient two concentration-time curves were drawn. Values are not dose normalised, unless otherwise stated. Dose normalised AUC_{0-12} values are normalised to 1000 mg MMF.

AcMPAG = acyl glucuronide metabolite of MPA, AUC = area-under-the-concentration-time-curve, CL = clearance, C_{max} = maximum concentration, LOQ = limit of quantification, MPA = mycophenolic acid, MPAG = glucuronide metabolite of MPA, T_{max} = time of maximum concentration after oral administration of mycophenolate mofetil, $t_{½}$ = half-life, V = volume of distribution.

Median apparent oral MPA CL was 56 L/h with a 3.4-fold between-patient variability. Median total MPA AUC_{0-12} normalised to 1000 mg MMF was 18 mg*h/L and median total MPA $t_{½}$ was 2.3 hours. Median total MPA pre-dose levels were 0.63 mg/L, indicating that the target trough level of 1.0 mg/L was not reached in most patients. Univariate analysis showed that after myeloablative conditioning, patients tended to have a higher median apparent oral MPA CL (60 L/h) than patients who underwent nonmyeloablative conditioning (43 L/h), but this difference was not statistically significant (p=0.12) and should be interpreted with caution, as there were only four patients treated with nonmyeloablative conditioning. No significant relationships could be identified between apparent oral CL and serum albumin levels (r_s=-0.02, p=0.93), between apparent oral CL and CsA dose (r_s=-0.24, p=0.36) or between apparent oral CL and CsA pre-dose level (r_s=-0.37, p=0.16). Apparent oral MPA CL correlated significantly with the number of highly albumin bound drugs a patient used during pharmacokinetic assessment (albumin binding of more than 90%, arbitrarily chosen) (r_s=0.50, p=0.05, figure 4a).

Median MPAG AUC_{0-12} and AcMPAG AUC_{0-12}, both normalised to 1000 mg MMF, were 430 mg*h/L and 3.2 mg*h/L respectively. Negative correlations between creatinine clearance (calculated according to Cockcroft and Gault) and the AUC_{0-12} values of these metabolites were identified (r_s=-0.74, p=0.001 for MPAG and r_s=-0.52, p=0.04 for AcMPAG).

Median dose normalised free MPA AUC_{0-12} was 224 µg*h/L. Median free fraction was 0.96%. A significant trend was observed towards higher MPA free fraction with the use of more highly albumin bound drugs: median free fraction was 0.8% with none, to 1.2% with two highly albumin bound drugs (r_s=0.51, p=0.05, figure 4b).

Due to the small sample size and the presence of many possible confounding factors, relationships between MMF pharmacokinetic parameters and acute GVHD or toxicity were not tested.

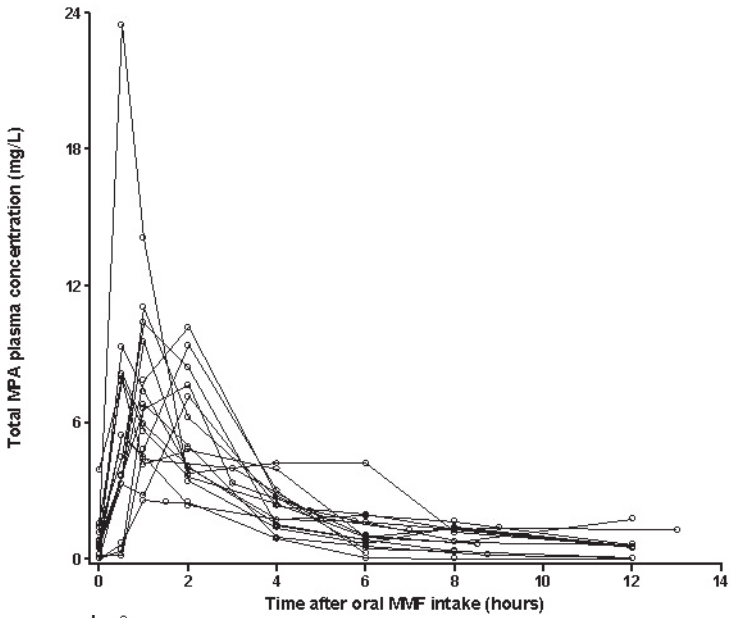

Figure 1 Individual concentration-time profiles of total mycophenolic acid (MPA) in 15 haematopoietic stem cell transplant recipients (excluding the patient presented as a case report). Two profiles are from one patient. Median mycophenolate mofetil dose was 1500 mg twice daily orally (range: 750-2000 mg twice daily).

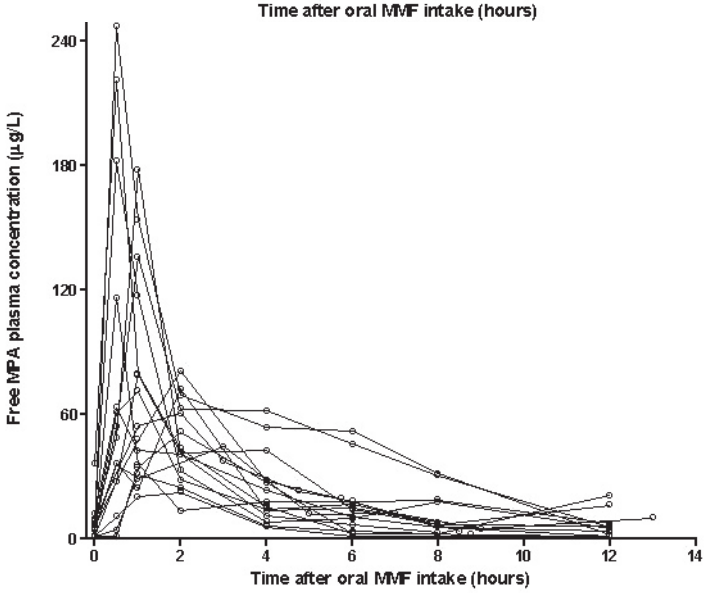

Figure 2 Individual concentration-time profiles of free mycophenolic acid (MPA) in 15 haematopoietic stem cell transplant recipients (excluding the patient presented as a case report). Two profiles are from one patient. Median mycophenolate mofetil dose was 1500 mg twice daily orally (range: 750-2000 mg twice daily).

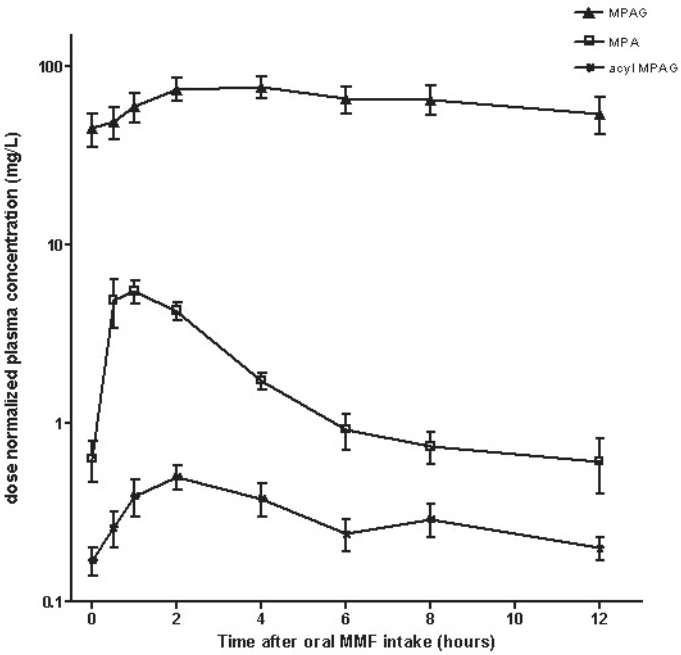

Figure 3 Mean dose normalised concentration-time curves with standard errors of total mycophenolic acid (MPA, open squares), MPA glucuronide metabolite (MPAG, closed triangles) and MPA acyl glucuronide metabolite (AcMPAG, stars). Data are from 15 patients and 16 concentration-time profiles (excluding the patient presented as a case report). Values are normalised to 1000 mg mycophenolate mofetil.

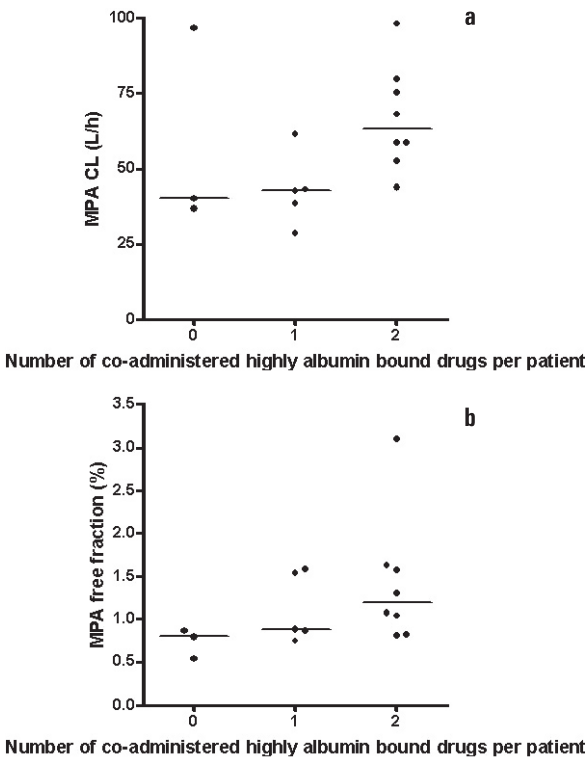

Figure 4 Correlation between the number of highly albumin bound drugs used by each patient during pharmacokinetic assessment and a) mycophenolic acid clearance (MPA CL), and b) MPA free fraction. Data are from 15 haematopoietic stem cell transplant recipients (excluding the patient presented as a case report). Two profiles are from one patient, explaining the 16 data points. Drugs used by one or more patients with an albumin binding of > 90% were: esomeprazole, itraconazole, amlodipine, valproic acid, temazepam, furosemide, bumetanide, spironolactone. r_s = Spearman's correlation coefficient. Horizontal lines represent the median of observations.

Case report

One patient with very low serum albumin levels (15 g/L) had two pharmacokinetic assessments shortly after each other (day 132 and day 139 after transplantation) to characterise the pharmacokinetics of MMF in a situation of hypoalbuminemia. This patient (age: 50 years) suffered from Hodgkin's Lymphoma, which was in partial remission at the time of HCT. Peripheral stem cells from a HLA-matched unrelated donor were transplanted after nonmyeloablatiave conditioning with fludarabine, TBI and ATG. Complete donorchimerism was reached within three months after transplantation. At the time of pharmacokinetic assessment, chronic extensive GVHD and multiple infections were present. GVHD was treated with oral prednisolon 75 mg twice daily. Apart from MMF, two other drugs with an albumin binding >90% were used on the first sampling occasion, namely esomeprazole and furosemide. On the second sampling occasion, three highly albumin bound drugs were used: spironolactone and bumetanide were added and furosemide was stopped. Creatinine clearance was 86 mL/min on the first occasion and 60 mL/min on the second occasion. The first concentration-time curve was drawn after oral intake of 500 mg MMF and resulted in a very low total MPA AUC_{0-12} of 4.6 mg*h/L (apparent oral MPA CL=109 L/h). Pre-dose total MPA level was 0.76 mg/L and maximum total MPA concentration was 0.90 mg/L. Total MPA concentrations were below 0.5 mg/L already 2 hours after dosing. Free MPA AUC_{0-12} was 171 µg*h/L resulting in a free fraction of 3.7%. MMF dose was adjusted based on the low pre-dose level to 1000 mg and one week later a second pharmacokinetic assessment was done after oral intake of MMF. Total MPA AUC_{0-12} was 1.8 mg*h/L (apparent oral MPA CL= 556 L/h), maximum total MPA concentration was 1.14 mg/L and total MPA concentrations could not be detected at 4, 6, 8 and 12 hours after MMF administration. Free MPA AUC_{0-12} was 647 µg*h/L and the free fraction was 36%.

DISCUSSION

MMF is increasingly being used in the treatment and prophylaxis of GVHD and the promotion of engraftment after HCT (10-12,15,18-20,29). The applied dose and dose interval of MMF are largely based on information derived from renal transplant studies, where extensive pharmacokinetic data have been gathered during the past ten years (3,30). Because of differences between solid organ transplant and HCT recipients as regards to a variety of aspects (e.g. renal function, albumin levels and co-medication) the pharmacokinetic relationships derived from organ transplantation may not be directly applicable to HCT.

This pilot study, including 15 HCT recipients, aimed at analysing the pharmacokinetics of MMF in HCT, when a dosing regimen based on knowledge from renal transplantation was applied, i,e twice daily administration of MMF with a total MPA target trough level > 1.0 mg/L.

The present analysis showed a median apparent oral MPA CL of 56 L/h, a median dose-normalised total MPA AUC_{0-12} of 18 mg*h/L, and a remarkably short median MPA $t_{½}$ of 2.3 h. These findings are in agreement with results from two other studies in patients treated with nonmyeloablative conditioning and unrelated donor HCT. The first study found mean total MPA AUC_{0-12} values of 23 and 24 mg*h/L and a $t_{½}$ of 3.0 and 3.8 h on day 7 and 21 respectively after HCT (n=34) (19). The second study observed a median total MPA AUC_{0-12} of 20 mg*h/L and a $t_{½}$ of 3.0 h (n=31) (12). In both studies, these values resulted from 15 mg/kg MMF twice daily orally. The same oral MMF dose produced a median total MPA AUC_{0-12} of 12 mg*h/L in four patients who received myeloablative conditioning and HCT from a sibling donor (21).

Data from renal transplant recipients within the first month after kidney transplantation receiving 1000 mg of MMF twice daily orally and concurrently treated with CsA show mean total MPA AUC_{0-12} values of 31 (31), 34 (32) and 39 (33) mg*h/L and apparent oral MPA CL values of 28 (33) and 35 (32) L/h. These data indicate that with the same MMF dose, apparent oral MPA CL in HCT recipients is almost doubled and MPA exposure is almost 50% lower than in renal transplant recipients. The values in HCT recipients would be considered sub-therapeutic in kidney transplant patients, where a therapeutic window of AUC_{0-12} levels between 30 and 60 mg*h/L has been adopted (22,34,35).

Median dose normalised exposure to free MPA was 224 µg*h/L. This value compares well with data from a study in 30 patients treated with nonmyeloablative conditioning and unrelated donor HCT, which found free MPA AUC_{0-12} to be 211 and 251 µg*h/L on day 7 and day 21 respectively (19). Also the value for free MPA-AUC_{0-12} of 247 µg*h/L found in 60 patients at week 1 after nonmyeloablative HCT is in close agreement with the results from the present study (20). Reported free MPA AUC_{0-12} mean values in renal transplantation are 600 (31) and 920 (32) µg*h/L. These data suggest that free MPA exposure is lower in HCT recipients.

Median exposure to AcMPAG AUC_{0-12} was 3.5 mg*h/L. This pharmacologically active metabolite has been associated with the development of gastrointestinal (GI) side effects that frequently occur with MMF therapy in renal transplant recipients (6). There are no suitable data available from renal transplant literature to compare with because the reported values for AcMPAG AUC_{0-12} are all from patients at least three months after transplantation, from patients concurrently treated with tacrolimus or from pediatric patients (36-38). The expectation is that exposure to AcMPAG will be comparable or lower than in renal transplant recipients as a result of the better renal function in HCT patients.

The present unselected population of fifteen HCT recipients had a heterogeneous composition with regard to underlying malignancies and conditioning regimens. Besides, the clinical condition between patients at the time of pharmacokinetic assessment was likely to vary as a result of the range of sampling occasions after HCT. Eight concentration-time curves were drawn within the first week after HCT, when patients may experience toxic adverse effects of the transplantation and the applied conditioning regimen, and eight profiles were drawn after the first week, when patients may have recovered but when acute GVHD may be present. The heterogeneity is likely to introduce significant variability in the pharmacokinetics of MMF (17). On the other hand, it makes the study population well representative of the common diversity of HCT recipients and, importantly, the predominant finding of low MPA exposure was present in nearly all patients. This indicates that the high apparent oral MPA CL in patients undergoing HCT is a very explicit effect. The clinical consequence is that higher MMF doses or more frequent dosing is necessary to attain optimised total and free MPA exposure. This is strengthened by the recent finding that low free MPA exposure was associated with an increased risk for GVHD (20). MMF administration three times a day has indeed been found to optimise total MPA exposure in patient undergoing HCT (19,21,39) which subsequently was predictive for a higher degree of donor T-cell chimerism in patients undergoing nonmyeloablative conditioning and unrelated donor HCT (19).

Due to the small sample size and the heterogeneity of the study population the causes of the high apparent oral MPA CL cannot be determined with the present data. The following observations however may have played a role, but are speculative and warrant further research. Firstly, the minor presence of EHC could have contributed. In renal transplant patients EHC is known to be responsible for 10 to 61% of total MPA exposure (3). Concentration-time profiles from this study however showed no secondary MPA peak concentrations (figure 1 and 2). High exposure to CsA may have contributed as it has been proven that CsA interrupts the EHC of MPA (40). A relationship between CsA dose or CsA trough

level and apparent oral MPA CL, however, could not be found. Further factors adding to the reduced contribution of EHC may be a damaged epithelium of the intestine caused by total body irradiation, chemotherapy and gut GVHD, or the presence of diarrhea at the time of pharmacokinetic assessment caused by gut GVHD or MMF related toxicity. Finally, the fact that all patients used 1 to 4 antibiotics at the time of pharmacokinetic assessment for selective decontamination of the gut flora, may have contributed (3,10).

A second aspect for the increased apparent oral MPA CL in HCT recipients may be the use of drugs with a high albumin binding (>90%) (figure 4a). In theory, the more highly albumin bound drugs a patient uses, the more competition may arise with MPA for albumin binding sites, thereby increasing MPA free fraction. The increased free fraction may in turn lead to an increased apparent oral MPA CL (30,32). Evidence for this hypothesis was provided by a significant positive correlation between the number of co-administered drugs with a high albumin binding and the MPA free fraction (figure 4b). The data from the case patient are also in agreement with the hypothesis, because this patient showed the highest apparent oral CL and free fraction values and used the highest number of drugs (three) with high albumin binding. The deviating data point in figure 4a for the patient with high MPA CL, but without the use of drugs with a high albumin binding, other than orally administered MMF, might be explained by the fact that this patient suffered from grade 3 mucositis and had diarrhea.

A third factor may be low bioavailability. Unfortunately, no intravenous data were available in this study to assess bioavailability, but it seems a plausible reason, because drug absorption is likely to be altered and variable due to gut toxicity after chemotherapy or gut GVHD. Evidence for this was provided by one study, which showed a median bioavailability of 63% in HCT recipients with a range of 13 to 161% (21). This median value is lower compared to values from renal transplantation (41). However, the observed low total MPA exposure can probably not be explained by reduced bioavailability alone, because low MPA exposure (both for AUC and pre-dose levels) was also found after intravenous administration of MMF to HCT recipients in previous studies (17,21,39).

Finally, low albumin levels may contribute to the high apparent oral MPA CL in HCT recipients. From renal transplantation it is known that low serum albumin levels lead to high apparent oral CL, presumably through an increased MPA free fraction (32,42). Although this analysis could not identify a relationship between serum albumin levels and apparent oral CL, the extremely low serum albumin level (15 g/L) and the high MPA free fraction values in the case patient may offer an explanation for the high apparent oral MPA CL observed in this patient. A previous study in patients treated with nonmyeloablative conditioning and unrelated donor HCT did find a positive correlation between total MPA AUC and serum albumin levels (19).

CONCLUSION

In conclusion, exposure to MPA after twice daily oral MMF administration in a case serie of stem cell transplant recipients representative for daily practice is low compared to reference values from renal transplantation as a result of high apparent oral MPA CL. More frequent MMF dosing is likely to optimise total MPA exposure. Whether this also leads to less GVHD needs to be investigated in pharmacokinetic-pharmacodynamic studies. With such studies, stem cell transplantation specific MPA target concentrations can be established and specific MMF dosing schedules be formulated.

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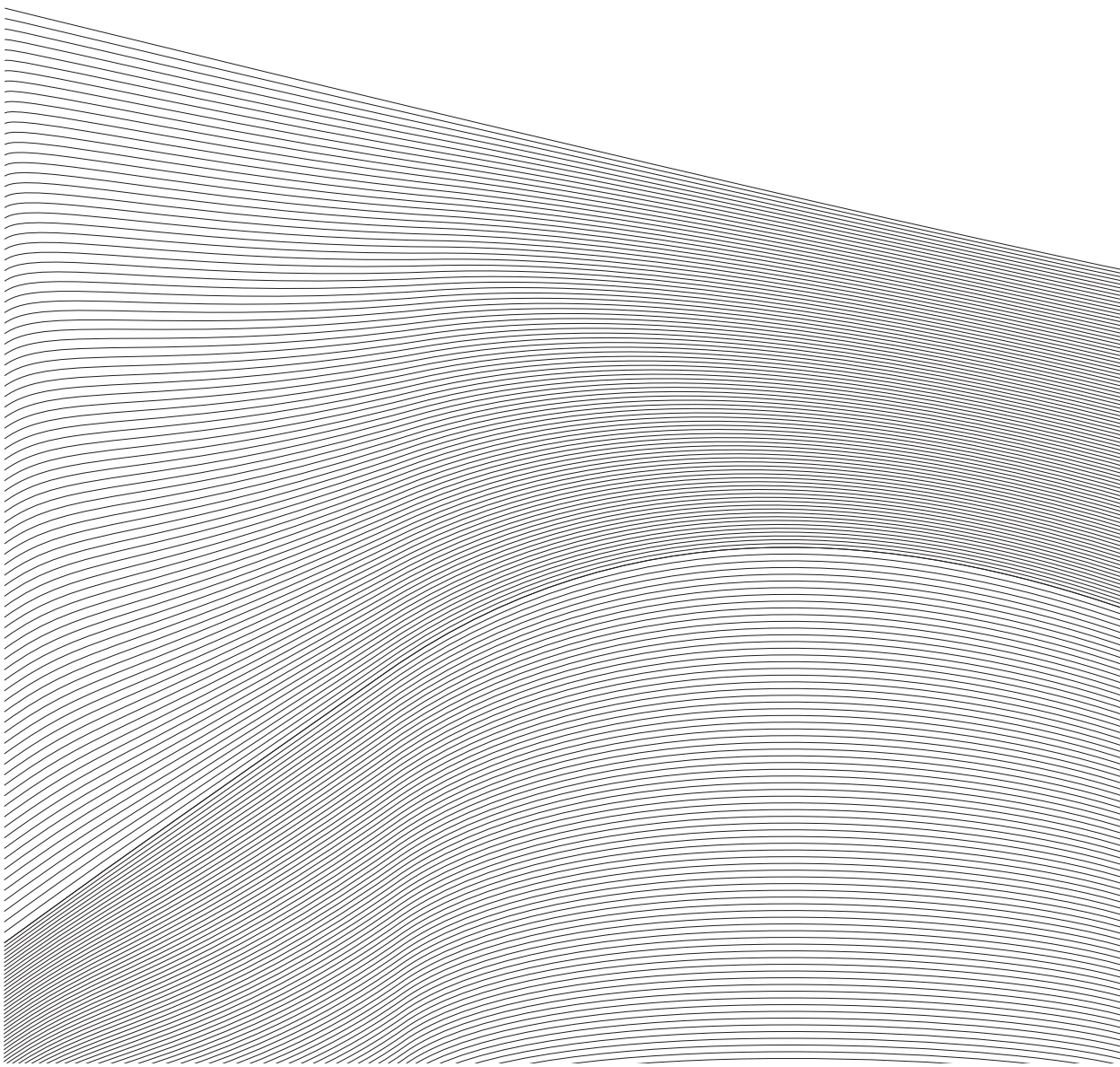

Chapter 5

GENERAL DISCUSSION

1 INTRODUCTION

Transplantation is a field in medicine where prevention is one of the corner stones of clinical care. The dosing regimens of immunosuppressive drugs applied after organ transplantation aim to balance between the prevention of under-immunosuppression, which may lead to acute rejection, and the prevention of over-immunosupression, which may cause toxicity, mainly represented by infections. Clearly, accurate and rational dosing of immunosuppressive drugs as well as the monitoring of immunosuppressive therapy is of life-saving importance.

It is, therefore, no surprise that the dose of all immunosuppressive drugs is somehow individualised. Azathioprine and corticosteroids are mainly dosed based on body weight. The dose of cyclosporine, and the newer compounds tacrolimus and sirolimus, are based on therapeutic drug monitoring strategies, where regular blood concentration measurements assure optimal exposure to the drug to minimise the risk of over- and underimmunosuppression. The exception is mycophenolate mofetil. Mycophenolate mofetil has been used so far with a dose recommendation of 1 gram twice daily in adult renal transplant recipients. This "one-dose-fits-all strategy" is thought to be one of the major advantages of the drug compared to other immunosuppressive agents, and makes it "easy to use without monitoring" [1]. Mycophenolate mofetil dose adjustments, if any, are based on the clinical situation of the patient with dose decreases when adverse effects occur and possibly dose increases in suspected cases of acute rejection. The important difference between this approach and therapeutic drug monitoring is that monitoring strategies *act* in order to prevent, instead of *reacting* to a clinical situation. Given the importance of prevention in transplantation, one may wonder whether the currently applied practice for mycophenolate mofetil leads to the best obtainable clinical outcomes. This is strengthened by 1) the observation that an area-under-the-concentration-time curve ($AUC_{0\text{-}12}$) of the active compound mycophenolic acid (MPA) below 30 mg*h/L is associated with an increased risk for acute rejection, 2) the 10-fold variability in MPA exposure between patients with standard mycophenolate mofetil doses, 3) the increase of MPA exposure over time despite a fixed mycophenolate mofetil dose and 4) drug-interactions between mycophenolate mofetil and concurrently administered immunosuppressive drugs. As explained in chapter 1.1, the combination of these four pharmacokinetic factors makes it unlikely that optimal MPA exposure, and, therefore, optimal immunosuppression is assured in all patients with a standard mycophenolate mofetil dose of 1 gram twice daily.

Despite the fact that the current practice for mycophenolate mofetil has been successful compared to azathioprine [2-4], it is to be expected that some of the adverse events and acute rejection episodes can be prevented in patients with suboptimal MPA exposure, through mycophenolate mofetil dose individualisation. The question thus arises how to identify (subsets of) candidate patients for dose individualisation. Two approaches could be suitable. The first one may be identification of patients through certain demographic characteristics that contribute to the variability in the pharmacokinetics of MPA, and the second one may be structural measurement of MPA concentrations to find patients with suboptimal MPA exposure. The hypotheses to be addressed in this thesis, as presented in chapter 1.2, were formulated around these two approaches. It is the aim of this chapter to discuss to what extent the combined main findings of the presented studies support the hypotheses that demographic patient factors and therapeutic drug monitoring are suitable to guide mycophenolate mofetil dose individualisation.

2 DEMOGRAPHIC FACTORS

The first hypothesis concerned demographic patient factors that (partly) explain the large observed variability in the pharmacokinetics of MPA. Such factors could potentially serve as a basis for mycophenolate mofetil dose individualisation. The following factors may be considered: co-medication, factors affecting MPA protein binding, time after transplantation, co-morbidity, and type of transplant.

2.1 Co-medication

In chapters 2.1, 2.2 and 2.4 population pharmacokinetic analyses were performed on large pharmacokinetic data sets to quantify the between- and within-patient variability in the pharmacokinetics of MPA and to identify patient factors that could explain this variability. One of the factors significantly affecting MPA pharmacokinetics was exposure to cyclosporine: the higher the exposure to cyclosporine, the higher the MPA clearance. A drug-interaction between MPA and cyclosporine has been found previously in clinical studies where patients treated with cyclosporine had lower MPA exposure than patients treated with sirolimus [5] or tacrolimus [6]. A study in rats suggested that cyclosporine interfered with the enterohepatic recirculation of MPA after having observed that the MPA $AUC_{6\text{-}12}$ was significantly lower in rats receiving cyclosporine compared to rats receiving tacrolimus [7]. An inhibitory effect of cyclosporine on the biliary excretion of the glucuronide metabolite of MPA (MPAG) could be proven in chapter 2.5, where there were no significant differences anymore between cyclosporine and tacrolimus treated rats who lacked the gene encoding for the multidrug resistance protein 2 (Mrp2). The function of Mrp2 is to excrete endogenous conjugates as well as conjugation products of drug metabolism into bile [8]. Inhibition of this enzyme by cyclosporine interrupts the enterohepatic recirculation of MPA, which leads to higher MPA clearance estimates as observed in chapters 2.1, 2.2 and 2.4.

The clinical consequence of the drug-interaction between MPA and cyclosporine was that patients who were exposed to cyclosporine exhibited significantly lower median dose normalised MPA $AUC_{0\text{-}12}$-values than patients not exposed to cyclosporine during the first year after renal transplantation as shown in chapter 2.2. Moreover, half of the patients who were concurrently treated with cyclosporine had MPA exposure, normalised to 1000 mg mycophenolate mofetil, below the recommended target $AUC_{0\text{-}12}$ level of 30 mg*h/L in the first week after transplantation in that study. This is of importance because a recent study found that optimal MPA exposure as early as day 3 after transplantation is associated with a lower incidence of acute rejection [9]. This justifies to recommend that in those patients who receive mycophenolate mofetil in combination with cyclosporine, the starting dose of mycophenolate mofetil should be 1500 mg twice daily instead of the currently recommended 1000 mg twice daily for optimal immunosuppression. Measurement of MPA exposure on day 3 after transplantation can further guide mycophenolate mofetil dosing. The hypothesis that the demographic factor "concurrent use of cyclosporine" provides a rationale for mycophenolate mofetil dose individualisation can thus be confirmed.

2.2 Factors affecting binding of MPA to plasma proteins

Besides exposure to cyclosporine, plasma albumin level and renal function are important determinants of MPA concentrations as was shown in chapters 2.1, 2.2, 2.4 and 2.6. In chapter 2.4, the relationship between creatinine clearance, as measure for renal function, and MPA clearance explained 19% within-patient variability and the correlation between plasma albumin level and MPA clearance explained 5% between- and 12% within-patient variability. Both effects on MPA clearance may be explained through an effect on MPA protein binding. Acidosis and uremia are associated with impaired renal function

and will decrease MPA binding to plasma albumin [10]. Moreover, accumulation of MPAG during renal impairment will displace MPA from its albumin binding sites [11]. Also low plasma albumin levels are likely to decrease MPA protein binding. As MPA is supposed to be a restrictively cleared drug, the increase of MPA free fraction leads to an increase of the amount of MPA available for glucuronidation and hence to a higher total MPA clearance. The result is a decrease in total MPA exposure, while exposure to free MPA is expected to remain the same [12,13]. However, conflicting results have been shown *in vivo* about the effect of renal function and plasma albumin level on free MPA concentrations [10,14-17]. The hypothesis that renal function and plasma albumin level affect total MPA exposure through an effect on MPA protein binding was tested in chapter 2.6, where a (semi)mechanistic model for the protein binding of MPA was developed. The results confirmed that renal function and plasma albumin level affect total MPA exposure through MPA protein binding: MPA free fraction increased with impaired renal function, high MPAG concentrations and low albumin levels. As a result, total MPA concentrations decreased, which is in accordance with the restrictive clearance concept. No effect of renal function or plasma albumin level could be shown on free MPA clearance.

With regard to individualisation of mycophenolate mofetil therapy based on changes in factors affecting binding of MPA to plasma proteins the following three aspects need to be taken into consideration. 1) The relationship between MPA exposure and the risk for acute rejection has been established for total MPA AUC_{0-12} and MPA predose level, but not for free MPA exposure [18-20]. Because total MPA concentrations are affected by renal function and plasma albumin level, dose adjustments are indicated in situations of poor renal function or low plasma albumin levels. 2) Exposure to the pharmacologically active free compound is not influenced by renal function or plasma albumin level, which would mean that dose adjustments are not indicated. 3) As shown in chapter 2.4, the same change in creatinine clearance or plasma albumin level does not necessarily lead to the same change in total MPA clearance in all patients. Besides, both relationships explained only a moderate amount of between- and within-patient variability in total MPA clearance. This would make creatinine clearance and plasma albumin level not suitable to directly serve as a basis for mycophenolate mofetil dose selection.

Based on these three aspects, the hypothesis cannot be confirmed that demographic factors that affect binding of MPA to plasma proteins offer a tool for mycophenolate mofetil dose individualisation. However, plasma albumin level and renal function can be of some guidance. Although a change in renal function or plasma albumin level does not directly provide an indication for dose adjustment, it does provide an indication to measure the total MPA concentration to check whether exposure has changed and whether dose adjustments are needed to maintain total MPA exposure above 30 mg*h/L. Importantly, an eventual dose increase will also increase free MPA exposure, and because high free MPA exposure has been linked to the occurrence of haematological adverse events [19,20], patients should be closely monitored. In a situation where a patient already has low leukocyte counts, measurement of free MPA concentrations may aid in the decision to adjust the mycophenolate mofetil dose. In such a situation, a free MPA AUC_{0-12} of 0.4 mg*h/L may be used as a cut-off value, as free MPA exposure above this value is expected to increase the risk for haematological side effects [19]. However, this cut-off value should be used with caution because it originates from a study in paediatric renal transplant patients in the early post-transplant phase [19]. A therapeutic window for free MPA exposure in adult renal transplant recipients where the risks for adverse events and acute rejection are minimised needs to be explored.

2.3 Time after transplantation

The increase of MPA AUC_{0-12} by 30 to 50% relative the immediate post-transplant period with a fixed mycophenolate mofetil dose is a well known feature of the pharmacokinetics of MPA [21]. In chapter 2.4 it was described that the increase in exposure is the result of a decrease in MPA clearance from 34 to 20 L/h during the first 165 days after renal transplantation. This decrease could be described, and can therefore be predicted, by improving renal function, increasing haemoglobin and plasma albumin level and decreasing cyclosporine predose concentration during that same period. The result of the gradual decrease in MPA clearance is that a subset of patients will have MPA exposure above the upper AUC_{0-12} limit of the recommended target window of 60 mg*h/L with standard mycophenolate mofetil doses of 1000 mg twice daily after 6 to 12 months after transplantation [22]. This is most likely in patients who have good renal function, normal plasma albumin level, and normal haemoglobin and in whom cyclosporine has been tapered or withdrawn. However, it is too early to recommend dose decreases after the first year in these patients for two reasons. The first one is that the recommended target window is only valid for the early phase after transplantation. Little is known about the optimal MPA concentrations after the first 6 months [23]. The second reason is that the increased MPA exposure may be very welcome in regimens in which cyclosporine is tapered or stopped to maintain sufficient immunosuppression, and in patients who tolerate the high MPA levels without toxicity, dose reductions may not be necessary. Consequently, before the hypothesis can be confirmed that "time after transplantation" is a suitable factor for mycophenolate mofetil dose individualisation, more information is necessary about the optimal MPA concentrations after the first 6 months after transplantation for favourable long-term outcomes.

2.4 Co-morbidity

In general, the presence of co-existent diseases may lead to alterations in the pharmacokinetics of a drug and may therefore cause the need for dose adjustments, especially those diseases that affect renal, hepatic, and cardiac function. In chapter 2.3 it has been investigated whether the pharmacokinetics of MPA are altered in renal transplant recipients who have co-existent diabetes mellitus. The results showed that patients with diabetes had an increased time of maximum MPA concentration, but further effects were absent. The delayed absorption of MPA in diabetic renal transplant recipients did not have consequences for the determination of MPA exposure with a limited sampling strategy. Because this analysis only included 7 patients with diabetes mellitus, the analysis was repeated in a large population pharmacokinetic meta-analysis (chapter 2.2) including 97 renal transplant recipients with diabetes mellitus. Again, no significant differences in the pharmacokinetics of MPA could be shown between diabetic and non-diabetic renal transplant recipients, other than a significant increased time of maximum MPA concentration.

Thus, for the co-morbidity diabetes mellitus no evidence was found to support the hypothesis that the mycophenolate mofetil dose should be adjusted on the basis of co-existent diabetes.

2.5 Type of transplant: haematopoietic stem cell transplantation

Following the success of mycophenolate mofetil in preventing acute rejection in renal transplantation, the drug is increasingly being used for other indications, for instance the prevention of graft-versus-host disease (GVHD) after haematopoietic stem cell transplantation (HCT) [24]. Because this is not a registered indication for the use of mycophenolate mofetil, dose finding studies are not performed and data on the pharmacokinetics of MPA after HCT are scarce. Consequently, mycophenolate mofetil

dose and dose interval applied in HCT recipients are largely based on pharmacokinetic data from renal transplant studies. This means that most HCT patients generally receive an oral starting mycophenolate mofetil dose of 15 mg/kg twice daily, often resulting in the standard mycophenolate mofetil dose recommended in renal transplantation of 1000 mg twice daily. In chapter 4 it is shown that this standard dose therapy leads to low MPA AUC_{0-12} values (10-35 mg*h/L per 1000 mg mycophenolate mofetil). These values would be regarded as subtherapeutic in renal transplantation. The low exposure is the result of a higher MPA clearance compared with reference values from studies in renal transplantation [10,25], and this would suggest that more frequent dosing may improve MPA exposure. Mycophenolate mofetil administration three times a day indeed has been found to optimise total MPA exposure in patient undergoing HCT [26-28], which in one study was predictive for a more favourable outcome (higher degree of donor T-cell chimerism) [26]. These results confirm the hypothesis that the type of transplant, in this case haematopoietic stem cells, is an important discriminator for mycophenolate mofetil dose selection. In general, it can be stated that the mycophenolate mofetil dosing principles as used in renal transplantation can not be blindly extrapolated to new indications for mycophenolate mofetil, for which there are hardly any pharmacokinetic data.

3 THERAPEUTIC DRUG MONITORING OF MPA

The second hypothesis was that therapeutic drug monitoring of MPA would be a suitable tool for mycophenolate mofetil dose individualisation, in that it would reduce a substantial amount of between-patient variability, resulting in target MPA exposure in virtually every patient, with a feasible sampling scheme.

3.1 Reducing variability between patients in exposure to MPA

The basic models resulting from the population pharmacokinetic analyses described in chapter 2.1 and 2.4 show a fare amount of (unexplained) between-patient variability in MPA clearance. Typical values were 31 L/h with 35% between-patient variability in chapter 2.1, and 27 L/h with 44% between-patient variability in chapter 2.4. The between-patient variability can be considered as large, because the range of MPA AUC values that will result from a fixed mycophenolate mofetil dose, will be much wider than the recommended target range [21]. Consequently, an important part of the renal transplant population, treated with a fixed dose of mycophenolate mofetil, will have under- or overexposure to MPA. Dose increases in patients with low MPA plasma concentrations, and dose decreases in patients with high concentrations are likely to reduce the between-patient variability. Candidates for dose adjustments may be identified through therapeutic drug monitoring. To compare the MPA exposure after a concentration-controlled (CC) dosing regimen with the MPA exposure after the current standard practice of fixed mycophenolate mofetil doses (FD), a simulation of both dosing regimens was performed for the first half year after transplantation (chapter 3.1). With a CC dosing regimen, significantly more patients had MPA AUC_{0-12} values within the recommended therapeutic window compared to the FD regimen already on day 7 after transplantation. To accomplish this, doses needed to be doubled in more than half of the patients, after having started with a dose of 1000 mg mycophenolate mofetil twice daily. Importantly, extremely high (>85 mg*h/L) and low (<20 mg*h/L) AUC_{0-12} values were prevented with a CC dosing regimen. It was concluded that therapeutic drug monitoring is expected to reduce between-patient variability in MPA exposure, resulting in target exposure in most patients.

3.2 When, how often, and which MPA exposure parameter to measure?

Given the conclusion that therapeutic drug monitoring is expected to optimise MPA exposure, the question arises when, how often and which MPA exposure parameter to measure, to indeed achieve and maintain target exposure. To answer this question, the amount of within-patient variability in MPA exposure has to be known. Large within-patient variability increases the likelihood that target exposure, once achieved, cannot be maintained for a long period of time, causing the need for frequent measurement of MPA concentrations. This may reduce the efficiency and clinical feasibility of therapeutic drug monitoring. In the basic population pharmacokinetic models presented chapter 2.1 and 2.4, the coefficient of variation (CV) values for within-patient variability in MPA clearance were respectively 30% and 34%. These values are comparable with the amount of within-patient variability in MPA exposure found in a previous study conducted in the maintenance phase after renal transplantation (≤30%), but studies performed in the initial period reported higher values (40 - 50%) [16,29]. The estimates from the initial period may have been overestimated, as it is unclear from these studies whether a correction was made for the structural increase of MPA $AUC_{0\text{-}12}$ over time.

In chapter 3.2, a clinical measure was developed to quantify the within-patient variability. After having divided MPA $AUC_{0\text{-}12}$ and predose levels for 9 sampling occasion into quartiles, within-patient variability was defined as a change of exposure from one quartile to another when going from one sampling occasion to the next. The advantage of this method is that small and clinically irrelevant changes in MPA exposure (one or no change of quartiles) can be distinguished from large fluctuations within a patient over time (two or three changes of quartiles), which might lead to gross over- or underexposure. Within-patient variability measured according to this method was found to be low, although the median score for predose level was significantly higher than for $AUC_{0\text{-}12}$ (6.0 versus 3.4 changes of quartiles of maximum 24 during the first 5 months after transplantation, respectively). The difference between $AUC_{0\text{-}12}$ and predose level only existed during the first two months after transplantation.

The important implication of the low within-patient variability is that exposure to MPA is expected to stable within a patient over time. Consequently, frequent measurements of MPA concentrations are not likely to be necessary. It may be speculated that one measurement of MPA exposure is indicated in the first week after transplantation to reduce between-patient variability and to get MPA exposure on target, and a second measurement would be beneficial several months after transplantation to compensate for the increase of MPA exposure over time. Unless major changes occur in patient condition or medication, more frequent measurements are not necessary as a consequence of the low within-patient variability. Furthermore, based on the results of chapter 3.2, MPA $AUC_{0\text{-}12}$ may be the preferred measure for MPA exposure (eventually estimated with a limited sampling strategy) during the first two months after transplantation on the basis of the lower within-patient variability compared with predose levels.

4 METHODOLOGICAL CONSIDERATIONS

In general, the basis for optimising dosing strategies are observations which may be collected in two different settings: a randomised controlled trial, or in, uncontrolled, observational studies. As a result of the stringent selection of patients and interventions in randomised controlled trials, the conclusions reached are often certain, and relatively simple methods can be used for data analysis [30]. Of course, the disadvantage is that extrapolation of the conclusions to other study populations may not be appropriate. Observational studies on the contrary are often much less well controlled, with the consequence that the obtained data are from a much more heterogeneous population. Therefore, the data from observational studies contain much more variability than data from randomised controlled trials, but are more representative for every-day practice. To reach unbiased and precise results during analysis of the heterogeneous data from observational studies, more complex methods for data analysis are necessary. The data analysis methods must often take into account repeated measures, counfounding, and imbalance in the number of data collected per subject [30]. Population pharmacokinetic modeling, as implemented in the software program nonlinear mixed effects modeling (NONMEM), provides a tool that satisfies these demands, and forms a pivotal part of the design of optimal dosing schedules in drug development [31].

In five out of the 9 described studies in this thesis (chapter 2.1, 2.2, 2.4, 2.6 and 3.1), population pharmacokinetic modeling was the method of choice for data analysis, for reasons of the heterogeneous nature of the data, the presence of confounders, the presence of repeated measures (number of sampling occasions per patient ranged from 1 to 9), and the imbalance in the collected data (collected number of concentration-time samples per patient ranged from 1 to 54).

A population pharmacokinetic model quantifies the central tendency of pharmacokinetic parameters in the studied population, and it quantifies the variability between patients in the typical population pharmacokinetic parameters. Variability in this matter refers to the probability distributions of deviations of individual values from the population mean [32]. Thus, by estimating variability, advantage is taken of the heterogeneity of the collected data. The quantification of variability offers the opportunity to estimate individual values of the pharmacokinetic parameters in an accurate way.

The population pharmacokinetic models in the chapters 1.2, 2.2 and 2.4 accounted for repeated measures by estimating, besides variability between patients, variability in the pharmacokinetic parameters within patients over time. This enables the estimation of different values of the pharmacokinetic parameters on different occasions within a patient. Estimation of within-patient variability further improves the accuracy of the estimated individual values for the pharmacokinetic parameters, whereas ignoring variability within patients over time can lead to biased estimates [33].

Pharmacokinetic variability between- and within-patients was not only quantified in the analyses in chapters 2.1, 2,2, 2.4 and 2.6, but it was also tried to be explained by establishing typical relationships between patient demographic features and individual pharmacokinetic parameter estimates, in a covariate analysis. When, for example, clearance varies with renal function, it can explain why one patient has another individual estimate for clearance than another patient, which may be important information for selecting the right dose for the right patient [32]. A caveat during covariate analyses is that different covariates can be correlated with each other, for instance when two covariates both increase over time after transplantation. This introduces the risk of the selection of covariates, which do not truly have a correlation with a pharmacokinetic parameter, i.e. confounding. To prevent confounding, a backward elimination procedure for covariate selection was performed in the analyses described in chapter 2.1, 2.2, 2.4 and 2.6, instead of a forward addition procedure, because the latter is known to be more vulnerable to selection of confounding factors [34]. Furthermore, a low alpha level

was chosen ($p<0.001$) during application of the likelihood ratio test to prevent selection of covariates, which are not truly correlated [35].

Chapters 2.2 and 2.4 were population pharmacokinetic meta-analyses, in which data sets from 6 different clinical studies were combined. These studies illustrate how the population pharmacokinetic approach is suitable to perform a powerful analysis of imbalanced data. In the different studies, the concentration-time data were collected with different sampling schemes and on different moments after transplantation. NONMEM allows for easy combination of these data [36]. Data are appropriately pooled across individuals and all data are fitted simultaneously to a pharmacokinetic model. As a result, the individual estimates for the pharmacokinetic parameters are less dependent on the number of samples per individual [37].

A disadvantage of population pharmacokinetic analyses is that every model is an oversimplification of reality [36,38]. As a result, every model will have a certain amount of error, which is estimated in a residual variability parameter. Because this parameter, which accounts for drug concentration measurement error, patient incompliance etc, is meant to reflect random variability, the error does not introduce a structural bias in the parameter estimates. Nevertheless, it is important to realize that results from population pharmacokinetic models are not perfect, and that model derived predictions for dose optimisation will differ to some extent from what will be seen in practice [36]. Another disadvantage is that population pharmacokinetic analyses are mathematically and statistically complex. This causes the need for specially trained data analysts, and often make the analyses time consuming. Nevertheless, a previous study showed that population pharmacokinetic analyses can be cost-effective [39]. A final disadvantage may be that the complexicity of the population pharmacokinetic methods constitute a barrier for clinicians to accept the results of a population pharmacokinetic study. The population pharmacokinetic approach is often regarded as a black box, which makes clinicians less eager to implement the results into practice. Therefore, there is a role for clinical pharmacologists to train clinicians in the general aspects of (population) pharmacokinetics, including its possibilities and its advantages. This is likely to open the way to a broader application of population pharmacokinetics in the design of clinical trials, the analysis of study results, and the selection of drug dosages in clinical practice.

With regard to the applied methodologies in this thesis, other than the population pharmacokinetic approach, two critical points ought to be made. The first one is that 6 out of the 9 studies had a retrospective nature. Although this allows for rapid and efficient analysis of large cohorts of patients over a long period of time (e.g. > 1 year in chapter 2.4), the information collected is often not as detailed as hoped for. When, for example, the time of drug-sample drawing is not recorded accurately, the estimate of the residual variability in the data analysis is likely to increase. In addition, a certain amount of data will be missing. In the analysis described in chapters 2.1, 2.2, 2.4 and 2.6, more than 10% of data were missing for some covariates. Different corrections were made for missing covariate data, like imputation with the population median value, but this is likely to introduce bias and may lead to underestimation of the influence of a covariate. Another correction, applied in chapter 2.4, was ignoring the concentration-time data from patients with missing covariate data. This method prevents the introduction of bias in the estimated effect of a covariate, but reduces the discriminating power of the analysis.

The second, and very important, critical note regards the end point used in every study in this thesis, namely exposure to MPA in plasma of patients. The relationship between MPA exposure and the risk for acute rejection in renal transplant patients concurrently treated with cyclosporine, has been firmly established as can be seen from table 1 in chapter 1.1. This also goes for the correlation between MPA exposure and adverse effects, although it is not as strong as the relationship with acute rejection (table 2, chapter 1.1). The relationships suggest that optimised exposure will lead to improved clinical

outcome. However, exposure to MPA is a surrogate endpoint. Consequently, the results with respect to how demographic factors or therapeutic drug monitoring can serve as a basis for mycophenolate mofetil dose individualisation to optimise MPA exposure, do not prove that dose individualisation will lead to less acute rejections and less toxicity. The conclusions of the presented studies must be interpreted as hypotheses that need confirmation in future trials. Therefore, the described and discussed results can not be directly implemented into clinical practice, but they form a rationale for the conduction and performance of new prospective studies, which aim to (further) improve the clinical use of mycophenolate mofetil through dose individualisation. A proposal for an optimised dosing scheme, based on the results of the research described in this thesis, to be tested in a prospective trial, is presented below.

5 INDIVIDUALISATION OF MYCOPHENOLATE MOFETIL THERAPY: A PROPOSAL

The results with regard to variability-explaining demographic factors, the extent of between-patient variability that can be reduced by therapeutic drug monitoring and the extent of within-patient variability in exposure to MPA, provide a basis for a proposal for an individualised mycophenolate mofetil dosing regimen. This proposal in presented in table 1. This individualised dosing regimen is designed to be clinically feasible, and at the same time to lead to optimal MPA exposure in most renal transplant patients as early as possible after surgery. This proposal is meant to be tested in prospective trials to prove that MPA exposure is indeed optimised and, more importantly, that it leads to improved clinical outcomes in terms of a lower incidence of biopsy proven acute rejection and less immunosuppressive related toxicity, like infections.

Table 1 Proposal for individualisation of the mycophenolate mofetil dosing regimen in renal transplant recipients

Event	Aim		Explanation in chapter/[ref]
1 Starting dose			
With concurrent cyclosporine	Total MPA AUC_{0-12} > 30 mg*h/L as early as possible in as many patients as possible	1500 mg twice daily	2.2; 3.1
With concurrent tacrolimus	Total MPA AUC_{0-12} > 30 mg*h/L as early as possible in as many patients as possible	1000 mg twice daily	
2 Day 3 after transplantation	Reduce between-patient variability and achieve total MPA AUC_{0-12} between 30 and 60 mg*h/L in every patient	Estimation of total MPA AUC_{0-12} with LSS with subsequent dose adjustment	3.1; 3.2 [10,22]
3 Stabilisation of renal function and albumin level (± month 1 after transplantation)	Maintain total MPA AUC_{0-12} between 30 and 60 mg*h/L	Estimation of total MPA AUC_{0-12} with LSS with subsequent dose adjustment	2.4; 3.2; 2.2
Notes			
- In case of tapering or withdrawal of cyclosporine (> month 3 after transplantation)			
Before tapering or withdrawal	Ensure total MPA AUC_{0-12} is > 50 mg*h/L for safe cyclosporine tapering	Estimation of total MPA AUC_{0-12} with LSS with subsequent dose adjustment	[40]
After tapering or withdrawal	Maintain target total MPA predose concentration (1.0-3.5 mg/L); higher concentrations are acceptable if tolerated	Measurement of total MPA predose concentration with subsequent dose adjustment	2.4; 3.2 [6]
- In case of sudden change in renal function or albumin level	Maintain total MPA AUC_{0-12} between 30 and 60 mg*h/L	Estimation of total MPA AUC_{0-12} with LSS with subsequent dose adjustment	2.1; 2.6
- In cases of MMF dose increases, not only total MPA AUC_{0-12} will increase, but also free AUC_{0-12}. This may increase the risk for haematologic adverse events. When these seem to occur	Keep free AUC_{0-12} < 0.4 mg*h/L	Measurement of free MPA AUC_{0-12} with subsequent dose adjustment	2.6 [15,19]

MPA = Mycophenolic acid; LSS = Limited sampling strategy.

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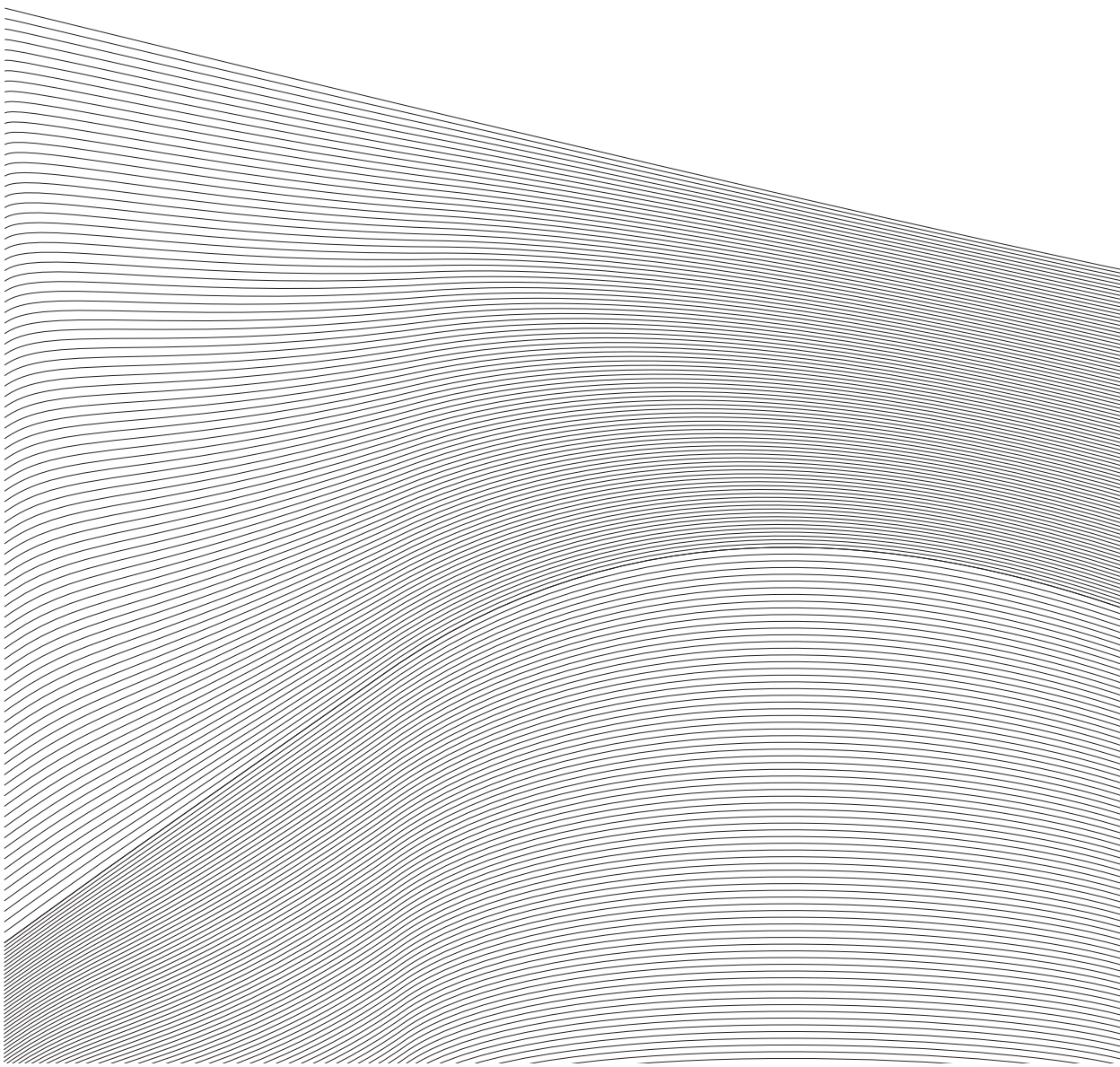
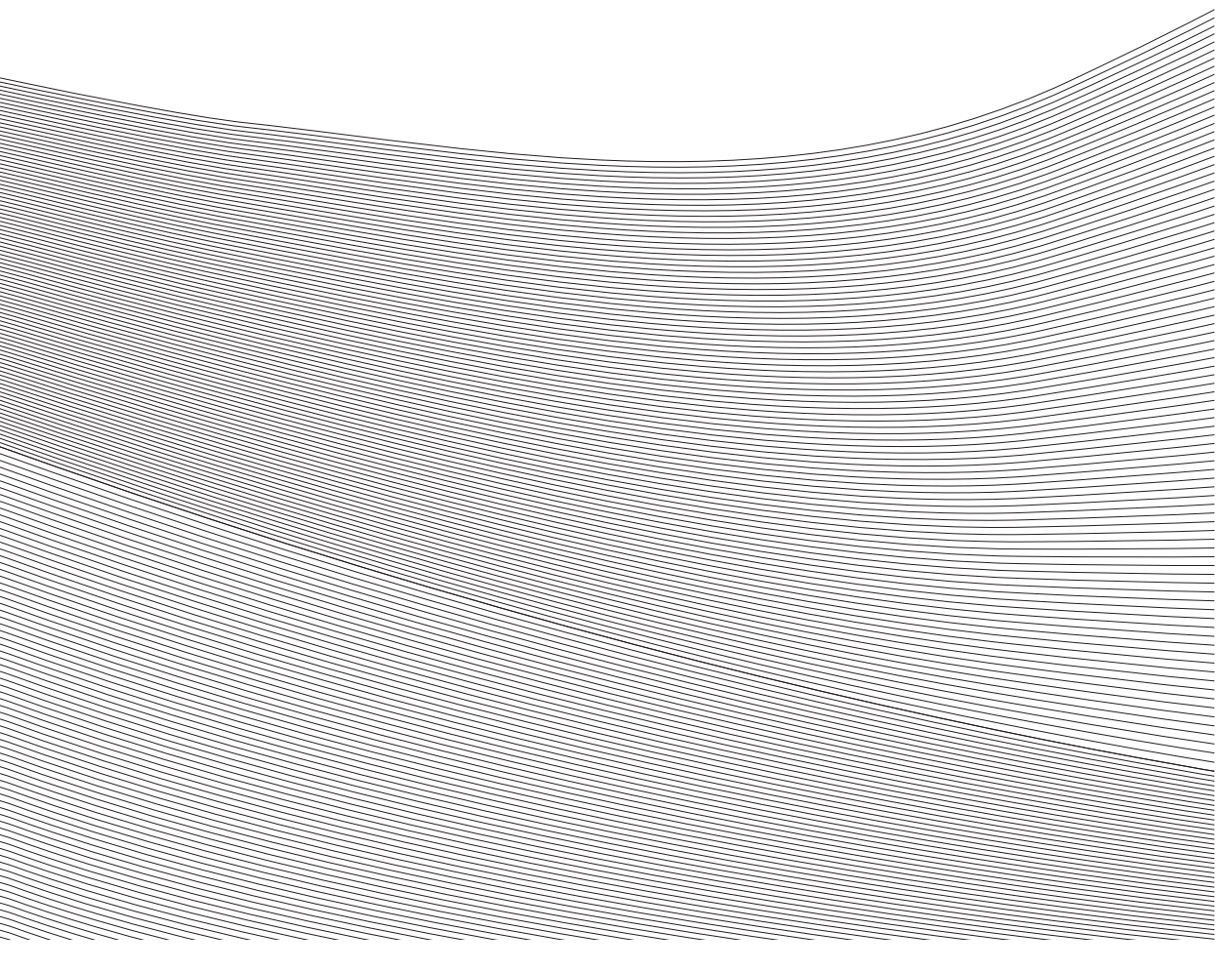


Chapter 6

SUMMARY



Chapter 6.1

Summary

The prodrug mycophenolate mofetil contains the active compound mycophenolic acid (MPA), which has immunosuppressive properties. It is used to prevent acute rejection after solid organ transplantation. In renal transplantation, the dose recommendation for mycophenolate mofetil is 1000 mg twice daily for adult patients. This fixed dose strategy for mycophenolate mofetil is remarkable in the field of transplantation as most other immunosuppressive drugs are used in an individualised dose, often based on drug concentration measurements. During the use of mycophenolate mofetil in the past ten years, data have become available which provide four reasons to question the justification of a fixed mycophenolate mofetil dose. The first reason is the existence of a concentration-effect relationship: the risk for acute rejection is lower when exposure to MPA is higher. This has led to the adoption of a target exposure range for MPA area-under-the-curve (AUC_{0-12}) values of 30 to 60 mg*h/L. The second reason is the large between-patient variability in MPA pharmacokinetics, reported to be more than 10-fold for MPA AUC_{0-12}. The third reason is that MPA exposure increases over time after transplantation despite a fixed dose. Finally, exposure to MPA is significantly influenced by the use of several other drugs. The result of these four factors is that with the use of a standard dose of mycophenolate mofetil, an important subset of renal transplant recipients will have MPA exposure outside the target range, and may therefore be at risk for acute rejection or toxicity. Individualisation of the mycophenolate mofetil dose is likely to improve exposure to MPA and may optimise clinical outcome.

The aim of this thesis was to develop recommendations about when and how to individualise the mycophenolate mofetil dose. Two hypotheses in this regard were addressed, formulated in **chapter 1.2.** The first was that demographic factors that contribute to the variability in the pharmacokinetics of MPA may serve as a rationale for mycophenolate mofetil dose individualisation (chapters 2.1 to 2.6, and 4.1). The second was that therapeutic drug monitoring of MPA provides a suitable tool for mycophenolate mofetil dose individualisation (chapters 3.1 and 3.2).

Chapter 1.1 extensively reviews the literature to introduce the reasons why individualisation of the mycophenolate mofetil dose may be beneficial. In addition, potential ways to individualise to mycophenolate mofetil dose are introduced. Based on the presented literature overview in chapter 1.1, the two hypotheses to be addressed in this thesis are formulated in chapter 1.2.

In **chapter 2.1**, a population pharmacokinetic model for MPA following oral administration of mycophenolate mofetil was developed, and relationships between patient factors and pharmacokinetic parameters were evaluated to quantify and explain variability in the phamacokinetics of MPA. For this purpose, MPA concentration-time data from 140 renal transplant patients collected on 9 occasions during the first 5 months after transplantation were retrospectively analysed with non-linear mixed effects modelling (NONMEM). The pharmacokinetics of MPA were best described by a two-compartment model with time-lagged first-order absorption. Population values for the first order absorption rate constant (k_a), apparent oral central volume of distribution (V1), and apparent oral clearance were 4.1 h^{-1}, 91 L and 33 L/h, respectively. The estimates for between-patient variability for k_a, V1, and apparent oral clearance were 111%, 91%, and 31%, respectively; estimates for within-patient variability for k_a, V1 and apparent oral clearance were 116%, 53% and 20%, respectively. Apparent oral MPA clearance correlated significantly with creatinine clearance, plasma albumin concentration, gender and daily cyclosporine dose ($p<0.001$), and these relationships could explain 11% and 33% of the observed between- and within-patient variability for apparent oral MPA clearance, respectively.

Chapter 2.2 tried to further elucidate whether renal function, plasma albumin level and the use of cyclosporine are suitable factors to serve as a basis for mycophenolate mofetil dose individualisation. This chapter was a population pharmacokinetic analysis including 1894 MPA concentration-time curves obtained from 468 renal transplant recipients with sampling occasions ranging from day 1 to day 3795 (>10 years) after transplantation. The results described in chapter 2.2 show that plasma albumin level and creatinine clearance as a measure for renal function are important predictors for apparent oral MPA clearance. The latter effect could also explain why patients with delayed graft function had a significantly lower median MPA AUC_{0-12} compared to those with immediate graft function during the first four days after transplantation (23 versus 33 mg*h/L respectively, $p<0.001$), and possibly also why black renal transplant patients exhibited lower MPA exposure during the first month after transplantation compared with Caucasian patients. Both observations were likely to be caused by a lower median creatinine clearance in patients with delayed graft function or in black patients. Nevertheless, renal function and plasma albumin level are not likely to be good candidates to serve directly as a basis for mycophenolate mofetil dose individualisation. The reason is that the same change in renal function or plasma albumin level will not have the same effect on apparent oral MPA clearance in every patient, as a result of the estimated large between-patient variability in the effect that renal function and plasma albumin level had on apparent oral MPA clearance (66% and 112%, respectively). A change in both variables merely provides an indication for therapeutic drug monitoring to check whether the mycophenolate mofetil dose needs to be adjusted in order to get or keep MPA exposure on target.

Furthermore, the results in chapter 2.2 show that the use of cyclosporine has an important impact on MPA exposure. Patients who were exposed to cyclosporine did not only have lower median dose normalised MPA AUC_{0-12}-values during the study follow-up compared with patients not exposed to cyclosporine, but half of the patients concurrently treated with cyclosporine had MPA exposure below the recommended target window in the first week after transplantation. Clinical outcome in these patients may be improved by starting with a mycophenolate mofetil dose of 1500 mg MMF twice daily instead of the currently recommended 1000 mg twice daily in the immediate post-transplant phase.

In **chapter 2.3**, it was investigated whether the presence of diabetes mellitus in renal transplant recipients had an impact on the pharmacokinetics of MPA. No significant differences in MPA exposure were found, but renal transplant patients with diabetes mellitus (n=6) had an increased median time to maximum MPA concentration (T_{max}) compared with renal transplant recipients without diabetes mellitus on day 11 after transplantation (1.59 h versus 0.67 h respectively, $p=0.04$). This result implies that patients with diabetes mellitus do not need an adjusted mycophenolate mofetil dose. However, the increased T_{max} may cause an underestimation of AUC values calculated from limited sampling strategies. When a limited sampling strategy developed and validated for non-diabetic renal transplant recipients was tested in the population of diabetic renal transplant patients, no significant bias was observed for the estimated AUC (mean bias of -1.5 mg*h/L with 95% confidence interval from -5.7 to 2.7 mg*h/L for 13 pharmacokinetic profiles from 7 diabetic renal transplant patients). The absence of a difference in the pharmacokinetics of MPA in diabetic renal transplant patients could be confirmed in the larger population in chapter 2.2 (97 renal transplant recipients with diabetes mellitus). Therefore, renal transplant recipients with diabetes mellitus do not need a different mycophenolate mofetil dosing regimen compared with patients without diabetes mellitus.

Chapter 2.4 aimed at describing the structural changes of apparent oral MPA clearance over time after transplantation. The data used were the same as in chapter 2.2. It was found that apparent oral clearance typically dropped from 34 L/h (coefficient of variation (CV) =3%) immediately after renal transplantation, to 20 L/h (CV=3%) 165 days (CV=12%) later. The same decrease in apparent oral MPA clearance, from 32 to 19 L/h, corresponded with a simultaneous change, representative for the first 6

months after transplantation, in creatinine clearance from 19 to 71 mL/min, in plasma albumin level from 35 to 40 g/L, in haemoglobin from 9.7 to 12 g/dL and in cyclosporine predose concentration from 225 to 100 ng/mL. These results indicate that by monitoring creatinine clearance, plasma albumin level, haemoglobin and cyclosporine predose concentration, the clinician can get a feeling about changes in apparent oral MPA clearance within a patient over time, which will mostly be a gradual decrease during the first 5 to 6 months after transplantation. Measurement of MPA exposure after the mentioned variables have stabilised in a patient, or after tapering of the cyclosporine dose, can identify those with high $AUC_{0\text{-}12}$ levels, and who may benefit from a mycophenolate mofetil dose reduction.

In **chapter 2.5**, the mechanism of the drug-interaction between MPA and cyclosporine was investigated. Three groups of ten rats, deficient for the multidrug resistance protein 2 (MRP2) gene, were orally treated for 6 days with either vehicle, cyclosporine or tacrolimus. In each group mycophenolate mofetil was added on day 7, and the pharmacokinetics of MPA and the glucuronide metabolite of MPA (MPAG) were analysed after single (day 7) and multiple doses (day 14). $AUC_{0\text{-}24}$ values for MPA and MPAG were not significantly different between cyclosporine- and tacrolimus-treated animals on both day 7 and 14. In a previous study in Lewis rats, not lacking the MRP2 gene, significant differences in MPA and MPAG exposure between rats cotreated with cyclosporine or tacrolimus were found. Therefore, it is suggested that cyclosporine-mediated inhibition of the biliary excretion of MPAG by the MRP2 transporter is the mechanism responsible for the interaction between cyclosporine and MPA.

In **chapter 2.6**, the mechanism of the effect of renal function and plasma albumin level on apparent oral total MPA clearance was studied. Total and free MPA concentrations, as well as total MPAG concentrations, were fitted simultaneously to several (semi-)mechanistic models. Data were collected from 88 renal transplant recipients on two occasions after transplantation (day 11 and 140). The model with the best fit was a 4 compartment model: a central and peripheral compartment for both unbound MPA and total MPAG with a link between the central compartments. Total MPA concentrations were modeled as the sum of free and bound MPA concentrations, where bound MPA concentration was the product of free concentration and a parameter representing linear protein binding. This model was in accordance with the theory for a restrictively cleared drug: a decrease in (the parameter for linear) protein binding leads to an increase of the free fraction and a decrease of total MPA concentrations, but leaves free concentrations unaffected. A decrease in plasma albumin level or renal function, or an increase in MPAG concentration correlated significantly with an increase in MPA free fraction. A significant relationship between the mentioned variables and apparent oral free MPA clearance could not be found. Thus, the results show that plasma albumin level and renal function influence total MPA exposure by affecting MPA binding to albumin, while an effect on free MPA exposure is absent. In addition, the model provides some evidence that MPAG displaces MPA from its albumin binding sites. Unfortunately, it is not possible to make sound mycophenolate mofetil dose recommendations based on these results: exposure to free MPA, regarded as the pharmacologically active moiety, is unaffected in patients with poor renal function or low plasma albumin level, and dose adjustments would not be indicated. However, total MPA exposure, for which a strong concentration-effect relationship exists, may be decreased with impaired renal function or low plasma albumin level, and this would imply that dose increases are necessary to minimise the risk for acute rejection.

In **chapter 3.1**, the usefulness of therapeutic drug monitoring was investigated with a computer simulation model. Two dosing regimens were compared: a fixed dosing regimen of 1000 mg mycophenolate mofetil twice daily (FD), and a concentration controlled (CC) dosing regimen, targeting MPA exposure at an $AUC_{0\text{-}12}$ level of 45 mg*h/L. The simulation was based on the Bayesian parameter estimates for apparent oral MPA clearance from 45 renal transplant recipients on 9 occasions during the first 5 months after renal transplantation who were also treated with cyclosporine, as determined

with the population pharmacokinetic model, described in chapter 2.1. The differences between both dosing regimens in percentage of patients with MPA exposure on target was compared. On day 7 after transplantation, significantly more AUC_{0-12} values were on target (AUC_{0-12} range 30-60 mg*h/L) in the CC group than in the FD group: 76% versus 13% respectively, $p<0.001$. To accomplish this improvement, a doubling of MMF dose was necessary in more than half of the patients. The occurrence of extremely high (>85 mg*h/L) or low (<20 mg*h/L) AUC values was prevented in the CC group. It was concluded that therapeutic drug monitoring of MPA is expected to bring a higher proportion of patients to adequate MPA exposure more rapidly.

In **chapter 3.2**, it was studied whether therapeutic drug monitoring would be feasible with regard to the expected frequency of MPA concentration measurements needed for optimal MPA exposure. For this purpose, the within-patient variability in MPA exposure was analysed, because variations over time within a patient cannot be controlled and may drive exposure away from the therapeutic window. This may cause the need for frequent monitoring. For 9 occasions during the first 5 months after transplantation, MPA AUC_{0-12} and predose values from 45 renal transplant recipients were divided into quartiles. When AUC_{0-12} or C_0 changed 1, 2 or 3 quartiles within a patient from one occasion to the next, a score of respectively 1, 2 or 3 points was assigned as a measure for within-patient variability. Within-patient variability measured according to this method was found to be low: for AUC_{0-12}, the median overall score was 3.4 of maximal 24. For C_0 measurements this score was significantly higher: 6.0 ($p<0.001$). The higher overall score for C_0 was explained by more quartile changes during the first weeks after transplantation. Based on the observations from this study, it is expected that therapeutic drug monitoring of MPA will be feasible, and that two measurements of MPA concentrations will be sufficient to optimise exposure: one measurement of MPA exposure, preferably by AUC_{0-12}, in the first week after transplantation to determine the optimal MMF dose, and a second measurement after 2 months to compensate for the increase in MPA exposure over time. Unless major changes in renal function or albumin levels occur, significant changes in MPA exposure are unlikely as a result of the low within-patient variability.

Mycophenolate mofetil is increasingly being used in the prophylaxis of graft-versus-host disease (GVHD) after haematopoietic stem cell transplantation, but there are only scarse data available about the pharmacokinetics of mycophenolate mofetil for this indication. Consequently, the dosing regimen is largely based on clinical experience in renal transplant patients. In **chapter 4**, the pharmacokinetics of mycophenolate mofetil in 15 haematopoietic stem cell transplant recipients was investigated, after application of a mycophenolate mofetil dosing regimen based on experience in renal transplantation, i.e. twice daily administration with a total MPA target predose level > 1.0 mg/L. Median mycophenolate mofetil dose was 1500 mg twice daily at the time of pharmacokinetic assessment, and sampling occasions ranged from day 7 to 26 after transplantation. Plasma concentrations of total MPA and its glucuronide metabolites, as well as free MPA were quantified. Median total MPA predose concentration, and total MPA AUC_{0-12} (normalised to 1000 mg MMF) were respectively 0.63 mg/L (range: < limit of quantification – 4.0 mg/L) and 18.0 mg*h/L (range: 10 – 35 mg*h/L). These low values are the result of a high median apparent oral MPA clearance of 56 L/h (range: 29 - 98 L/h). This value is almost double the reported values for apparent oral MPA clearance from renal transplant recipients (e.g. 28 and 35 L/h). The observed MPA exposure in haematopoietic stem cell transplantation recipients would be considered sub-therapeutic in kidney transplant patients. Exposure may be improved by higher or more frequent MMF dosing. However, pharmacokinetic-pharmacodynamic studies are necessary to formulate MPA target concentrations and mycophenolate mofetil dosing schedules, specific for haematopoietic stem cell transplantation.

Chapter 5 discusses to what extent the combined main findings of the presented studies are in agreement with the specific hypotheses formulated in chapter 1.2. In addition, some comments are given with regard to the applied methodology in the different chapters, with special attention to population pharmacokinetic modeling. Finally, an individualised mycophenolate mofetil dosing regimen is proposed for renal transplant recipients (table 1 of chapter 5). The proposal compromises 1) a mycophenolate mofetil starting dose of 1500 mg twice daily when mycophenolate mofetil is combined with cyclosporine, 2) a measurement or estimation of MPA $AUC_{0\text{-}12}$ on day 3 after renal transplantation to reduce between-patient variability and achieve target MPA exposure, and 3) a measurement or estimation of MPA $AUC_{0\text{-}12}$ when plasma albumin level and renal function have stabilised (presumably around month 1 after transplantation). Further measurements of MPA exposure are proposed in case cyclosporine is tapered or withdrawn, or in case a sudden change in renal function or plasma albumin level occurs.

Chapter 6.2

Samenvatting voor niet-ingewijden

1 INLEIDING

Patiënten die een orgaantransplantatie hebben ondergaan gebruiken geneesmiddelen die het afweersysteem onderdrukken. Dat is nodig omdat het afweersysteem het nieuwe orgaan als lichaamsvreemd ziet, waardoor, net als bij een bacterie- of virusinfectie, een reactie op gang komt die al wat lichaamsvreemd is zal proberen te vernietigen. Een dergelijke reactie van het afweersysteem gericht tegen het getransplanteerde orgaan wordt een afstotingsreactie genoemd. Wanneer het afweersysteem onderdrukt wordt met behulp van geneesmiddelen, zal het afweersysteem veel minder snel een afstotingsreactie ontwikkelen tegen het lichaamsvreemde orgaan. Op deze manier wordt geprobeerd om te voorkomen dat het gedoneerde orgaan verloren gaat. Voorbeelden van geneesmiddelen die in verschillende combinaties gebruikt worden om het afweersysteem te onderdrukken bij transplantatie patiënten om de kans op een afstotingsreactie zo klein mogelijk te maken, zijn ciclosporine, prednison, azathioprine, tacrolimus en mycofenolaat mofetil. Voor welke combinatie wordt gekozen is afhankelijk van een aantal patiëntkenmerken, bijvoorbeeld het vooraf ingeschatte risico op een afstotingsreactie of de aanwezigheid van contra-indicaties voor één van de geneesmiddelen, en de voorkeur die men heeft in het betreffende medisch centrum. Dit proefschrift richt zich op mycofenolaat mofetil, afgekort als MMF. MMF onderdrukt het afweersysteem doordat het de vermenigvuldiging van witte bloedcellen remt die normaal gesproken in grote aantallen nodig zijn om lichaamsvreemde weefsels te kunnen vernietigen. Het gevolg is dat het afweersysteem minder snel tot een afstotingsreactie komt tegen het getransplanteerde orgaan.

Na de introductie van MMF in 1995 is het geneesmiddel vooral toegepast bij niertransplantaties. Gebleken is dat de kans op het krijgen van een afstotingsreactie bij gebruik van MMF in combinatie met ciclosporine en prednison ongeveer twee keer zo klein is als wanneer azathioprine in plaats van MMF gebruikt wordt: < 20% versus respectievelijk ongeveer 40%. MMF is dus een succesvol geneesmiddel.

Gedurende het eerste decennium waarin ervaring is opgedaan met MMF zijn er gegevens beschikbaar gekomen die erop wijzen dat de kans op het krijgen van een afstotingsreactie nog verder teruggedrongen zou kunnen worden. Het gaat om de volgende vier punten, die uitgebreid beschreven worden in hoofdstuk 1.1:

1. Het is gebleken dat de kans op een afstotingsreactie kleiner is, naarmate de mycofenolzuur blootstelling in het bloed hoger is. Mycofenolzuur is de verschijningsvorm van MMF in het bloed die zorgt voor de feitelijke remming van de vermenigvuldiging van witte bloedcellen. Vastgesteld is dat de blootstelling aan mycofenolzuur in het bloed[1] idealiter boven de 30 mg*h/L moet liggen om het risico op een afstotingsreactie bijwerkingen zo klein mogelijk te laten zijn.
2. Het is gebleken dat de blootstelling aan mycofenolzuur in het bloed sterk verschilt van patiënt tot patiënt ondanks dat alle patiënten dezelfde dosering MMF kregen, zoals aanbevolen door de fabrikant, namelijk 1 gram twee maal per dag. Met deze dosering varieert de blootstelling aan mycofenolzuur in het bloed tussen patiënten van 10 tot 100 mg*h/L.
3. De mycofenolzuur blootstelling in het bloed van een patiënt stijgt gedurende de eerste maanden na niertransplantatie, zelfs bij constante dosering MMF.
4. Door gelijktijdig gebruik van verschillende geneesmiddelen met MMF kan de blootstelling aan mycofenolzuur in het bloed beïnvloed worden. Hierdoor kan de ene patiënt een hogere blootstelling hebben dan de andere patiënt, terwijl ze dezelfde dosering MMF gebruiken.

[1] De blootstelling aan een geneesmiddel in het bloed wordt bepaald door gedurende de periode tussen twee doseringen op minstens acht verspreide tijdstippen de concentratie van het geneesmiddel in het bloed te meten, om vervolgens door de meetpunten een lijn te trekken en daar de oppervlakte onder te berekenen. Deze maat staat bekend als de oppervlakte-onder-de-concentratie-tijd-curve, afgekort als AUC.

Het gevolg van bovenstaande vier punten is dat met de gangbare dosering van MMF van twee maal per dag 1 gram een deel van de niertransplantatie patiënten een te lage mycofenolzuur blootstelling heeft in het bloed (< 30 mg*h/L). Deze patiënten hebben een onvoldoende onderdrukt afweersysteem en dus een groter risico op een afstoting van de nieuwe nier. Een ander deel van de patiënten zou een te hoge mycofenolzuur blootstelling kunnen hebben. Algemeen wordt hierbij een mycofenolzuur blootstelling in het bloed groter dan 60 mg*h/L aangehouden. Bij een hogere blootstelling dan deze waarde is er geen verdere daling van de kans op een afstotingsreactie.

Het idee is nu dat het aantal afstotingsreacties verlaagd kan worden door ervoor te zorgen dat alle niertransplantatie patiënten een blootstelling aan mycofenolzuur in het bloed hebben die tussen de 30 en 60 mg*h/L ligt. Om dat te bereiken zal een deel van de niertransplantatie patiënten een hogere MMF dosering nodig hebben dan twee maal per dag 1 gram, terwijl een ander deel van de patiënten juist met een lagere dosering op een goede mycofenolzuur blootstelling in het bloed kan uitkomen. De vraag is echter welke patiënten (bijvoorbeeld met betrekking tot leeftijd, gewicht, geslacht, transplantaatfunctie) meer of juist minder MMF moeten hebben, en hoe groot die bijstelling dan moet zijn? Hierop wordt ingegaan in hoofdstuk 1.1.

2 DOELSTELLING EN HYPOTHESES VAN DIT PROEFSCHRIFT

Het doel van dit proefschrift, zoals verwoord in hoofdstuk 1.2, is om aanbevelingen te ontwikkelen over welke MMF dosering op welk moment na transplantatie het beste past bij een individuele patiënt om de ideale mycofenolzuur blootstelling in het bloed te bereiken van 30 - 60 mg*h/L. Twee hypotheses zijn onderzocht. De eerste hypothese was dat de ideale blootstelling aan mycofenolzuur in het bloed in meer patiënten bereikt kan worden door de MMF dosering toe te spitsen op individuele patiëntkenmerken, zoals onder andere geslacht, gewicht, kwaliteit van het functioneren van het transplantaat of gelijktijdig gebruik van MMF met verschillende geneesmiddelen (prednison, ciclosporine etc.). Als bijvoorbeeld blijkt dat veel mannen een mycofenolzuur blootstelling hebben die kleiner is dan 30 mg*h/L, terwijl de meeste vrouwen wel een adequate blootstelling hebben met de gangbare MMF dosering, dan hebben mannen blijkbaar een hogere dosering nodig, en is geslacht een geschikt patiëntkenmerk om de MMF dosering op te baseren. De tweede hypothese was dat de ideale blootstelling aan mycofenolzuur in het bloed in meer patiënten bereikt kan worden door periodiek de blootstelling aan mycofenolzuur in het bloed te meten als basis voor aanpassing van de MMF dosering. Dit wordt therapeutic drug monitoring genoemd. Hierbij wordt de dosering MMF verhoogd als in een patiënt een te lage mycofenolzuur blootstelling gemeten is, en wordt de dosering verlaagd als in een patiënt een te hoge blootstelling gemeten is.

3 HYPOTHESE 1: IDEALE MYCOFENOLZUUR BLOOTSTELLING KAN IN MEER PATIËNTEN BEREIKT WORDEN DOOR DE MMF DOSERING TOE TE SPITSEN OP PATIËNTKENMERKEN

De eerste hypothese wordt onderzocht in hoofdstukken 2.1 tot en met 2.4. In hoofdstuk 2.1 zijn gegevens over de blootstelling aan mycofenolzuur, verzameld gedurende het eerste half jaar na transplantatie, van 140 niertransplantatiepatiënten onderzocht. Het doel was te beschrijven hoe sterk de blootstelling aan mycofenolzuur in het bloed varieerde tussen deze patiënten, uitgaande van het gegeven dat elke patiënt dezelfde MMF dosering kreeg. De blootstelling aan mycofenolzuur tussen patiënten varieerde tussen de 13 en 54 mg*h/L. Ook is bekeken in welke mate de blootstelling aan mycofenolzuur varieerde binnen een en dezelfde patiënt in de tijd. Het bleek dat de mycofenolzuur blootstelling in het bloed in de meest extreme patiënt op het ene moment 20 mg*h/L kon zijn, terwijl enkele weken later de blootstelling in diezelfde patiënt en met dezelfde MMF dosering 46 mg*h/L kon zijn.

3.1 Oorzaken voor de variërende blootstelling aan mycofenolzuur tussen patiënten

In hoofdstuk 2.1, maar vooral in hoofdstuk 2.2 en hoofdstuk 2.3, zijn de oorzaken onderzocht voor de variërende blootstelling aan mycofenolzuur tussen niertransplantatie patiënten. Het is belangrijk om deze oorzaken te kennen, omdat ze mogelijk zouden kunnen dienen als basis voor de selectie van de juiste individuele MMF dosering om ideale blootstelling aan mycofenolzuur te bereiken.

3.1.1 Invloed van het functioneren van de nier, de albumine concentratie en het gebruik van ciclosporine

Voor de studie beschreven in hoofdstuk 2.2 zijn gegevens over de mycofenolzuur blootstelling en patiëntkenmerken (zoals geslacht, gewicht, functioneren van de nieuwe nier, gelijktijdig gebruik van andere geneesmiddelen) van 468 niertransplantatie patiënten over een periode van 10 jaar met elkaar gecombineerd. Uit de analyse kwam naar voren dat naarmate de getransplanteerde nier beter functioneerde, de blootstelling aan mycofenolzuur hoger werd. Ook bleek dat wanneer een patiënt een lage concentratie albumine[2] in zijn of haar bloed had, de blootstelling aan mycofenolzuur lager was. Op basis van deze bevindingen zou geconcludeerd kunnen worden dat niertransplantatie patiënten met een slecht functionerende nier en/of patiënten met een lage hoeveelheid albumine in hun bloed een hogere dosering MMF moeten hebben voor een ideale mycofenolzuur blootstelling. Deze conclusie gaat echter maar gedeeltelijk op, omdat uit het onderzoek in hoofdstuk 2.2 ook naar voren kwam dat beide factoren niet in alle patiënten eenzelfde effect op de mycofenolzuur blootstelling hadden. Dat wil zeggen dat een patiënt met een slecht functionerende nier niet per definitie een lage blootstelling aan mycofenolzuur heeft. Omdat de functie van de nieuwe nier en de concentratie albumine in het bloed in een groot deel van de patiënten wél invloed zal hebben op de mycofenolzuur blootstelling en bij slechts een minderheid niet, is het raadzaam om bij afwijkende waarden van deze factoren, of bij plotse veranderingen in deze factoren, de mycofenolzuur blootstelling in het bloed te meten. Op deze manier kunnen die patiënten worden geselecteerd die inderdaad een te lage blootstelling hebben en die daarom in aanmerking komen voor aanpassing van de MMF dosering om de blootstelling tussen de 30 en 60 mg*h/L te krijgen.

[2] Albumine is een lichaamseigen eiwit dat dient als transportmiddel van allerlei lichaamseigen en lichaamsvreemde stoffen door het bloed.

Naast de kwaliteit van het functioneren van de nier en de hoeveelheid albumine in het bloed bleek uit de analyse beschreven in hoofdstuk 2.2 dat ook het gebruik van ciclosporine invloed heef op de mycofenolzuur blootstelling: naarmate in het bloed meer ciclosporine aanwezig is, wordt de blootstelling aan mycofenolzuur lager. Als gevolg hiervan blijkt dat de helft van de niertransplantatie patiënten die gelijktijdig MMF en ciclosporine gebruikten, in de eerste week na transplantatie een mycofenolzuur blootstelling hadden die kleiner was dan 30 mg*h/L. Daarom zou, ter voorkoming van een te lage mycofenolzuur blootstelling in niertransplantatie patiënten die gelijktijdig behandeld worden met ciclosporine en MMF, de startdosering van MMF twee maal daags 1.5 gram moeten zijn in plaats van de gebruikelijke 2 maal daags 1 gram.

3.1.2 Invloed van diabetes mellitus

In hoofdstuk 2.3 is onderzocht of het hebben van diabetes mellitus (suikerziekte) invloed heeft op de variërende blootstelling aan mycofenolzuur tussen niertransplantatie patiënten. De invloed van diabetes mellitus is onderzocht, omdat deze aandoening regelmatig voorkomt bij niertransplantatiepatiënten. Uit gegevens van 7 diabetische niertransplantatie patiënten die vergeleken zijn met gegevens van 129 niet-diabetische niertransplantatie patiënten bleek dat de blootstelling aan mycofenolzuur tussen beide groepen niet verschillend was. Wel is gevonden dat de maximale mycofenolzuur concentratie in het bloed later bereikt werd bij diabetische niertransplantatie patiënten dan bij niet-diabetische niertransplantatie patiënten. Vermoedelijk wordt dit veroorzaakt door een vertraagde maaglediging, wat een bekend verschijnsel is bij diabetes mellitus, waardoor de opname van mycofenolzuur in het bloed vanuit de darm later plaatsvindt. Het effect op de maximale mycofenolzuur concentratie kon bevestigd worden in een vervolgonderzoek in een grotere groep van 97 niertransplantatie patiënten met diabetes mellitus (hoofdstuk 2.2)

3.1.3 Veranderingen in de tijd

Hoofdstuk 2.4 had tot doel om de veranderingen in de blootstelling aan mycofenolzuur gedurende de eerste maanden na niertransplantatie bij gelijkblijvende MMF dosis te beschrijven en te verklaren. Hierbij is onderzocht welke patiëntkenmerken, die mogelijk een rol spelen bij de variërende blootstelling aan mycofenolzuur, veranderen in de tijd na transplantatie. De gegevens die voor deze analyse gebruikt zijn, zijn dezelfde als in hoofdstuk 2.2. Gemiddeld gezien neemt de blootstelling aan mycofenolzuur in het bloed van niertransplantatie patiënten toe van 29 mg*h/L direct na transplantatie naar 50 mg*h/L 165 dagen na transplantatie, uitgaande van een MMF dosering van twee maal daags 1 gram gedurende de hele periode. De toename in de blootstelling aan mycofenolzuur gedurende de eerste maand na transplantatie loopt parallel in de tijd met, en kan vermoedelijk verklaard worden door een verbetering van de functie van de getransplanteerde nier en een stijging van de hoeveelheid albumine in het bloed[3]. De stijging in de mycofenolzuur blootstelling gedurende maand 2 tot 6 na transplantatie loopt voornamelijk parallel met een afname in de ciclosporine blootstelling[4]. De toename van de mycofenolzuur blootstelling in de tijd kan dus gevolgd worden door het meten van de nierfunctie, de albumine concentratie en de ciclosporine concentratie in het bloed. Dit zijn allemaal variabelen die routinematig ter controle in niertransplantatie patiënten gevolgd worden. Naar aanleiding van de analyse in hoofdstuk 2.4 kan worden aanbevolen om de blootstelling aan mycofenolzuur in het bloed te meten wanneer de albumine concentratie en de nierfunctie gestabiliseerd zijn, wat meestal ongeveer een maand na transplantatie het geval is. In een aantal patiënten zal dan de mycofenolzuur blootstelling groter zijn dan 60 mg*h/L en kan een verlaging van de MMF dosering overwogen worden.

[3] Verbetering van de nierfunctie en stijging van de albumine hoeveelheid in het bloed over de tijd zijn ontwikkelingen die karakteristiek zijn voor de herstellende niertransplantatie patiënt na een ingrijpende operatie.

[4] Afname van de blootstelling aan ciclosporine is het gevolg van stapsgewijze verlaging van de ciclosporine dosering die in het algemeen gedaan wordt omdat gebleken is dat minder onderdrukking van het afweersysteem noodzakelijk is na de eerste fase na de transplantatie.

Samengevat kan de variërende blootstelling aan mycofenolzuur tussen niertransplantatie patiënten, die bestaat ondanks gelijkblijvende MMF dosering, voor een deel verklaard worden door verschillen tussen patiënten in kwaliteit van het functioneren van de nier, de hoeveelheid albumine in het bloed en de mate van blootstelling aan ciclosporine. Daarnaast lijken deze drie factoren bij te dragen aan het verschijnsel dat de blootstelling aan mycofenolzuur in het bloed toeneemt bij niertransplantatie patiënten tijdens de eerste maanden na transplantatie. De ideale blootstelling aan mycofenolzuur in het bloed (tussen de 30 en 60 mg*h/L) kan in meer patiënten bereikt worden door de MMF dosering toe te spitsen op het al dan niet gelijktijdige gebruik van ciclosporine: indien MMF gecombineerd wordt met ciclosporine is een MMF startdosis van twee maal daags 1.5 gram aan te bevelen direct na de transplantatie; wanneer MMF niet gecombineerd wordt met ciclosporine (bijvoorbeeld wanneer er een contra-indicatie aanwezig is) kan gestart worden met de gangbare dosis van twee maals daags 1 gram. Na een maand, als de nierfunctie en de albumine concentratie in het bloed gestabiliseerd zijn, zal in een deel van de patiënten de blootstelling aan mycofenolzuur gestegen zijn, ondanks gelijkblijvende MMF dosering. Meting van de mycofenolzuur blootstelling in het bloed is dan zinvol om die patiënten te identificeren die inderdaad een hoge blootstelling hebben en waar de dosering van MMF verlaagd kan worden.

3.2 Onderzoek naar de oorzaak van de invloed van de transplantaat functie, de albumine concentratie en het gebruik van ciclosporine

Hoofdstukken 2.5 en 2.6 richten zich op de vraag hoe het komt dat de transplantaat functie, de albumine concentratie en het gebruik van ciclosporine invloed hebben op de mycofenolzuur blootstelling.

3.2.1 De oorzaak van de invloed van het gebruik van ciclosporine

In hoofdstuk 2.5 is de reden onderzocht van de lage mycofenolzuur blootstelling in niertransplantatie patiënten die behandeld worden met de combinatie van MMF en ciclosporine. Voor dit onderzoek is gebruik gemaakt van genetisch gemodificeerde ratten. In deze ratten ontbreekt het enzym "multidrug resistance protein 2" (MRP2). De volgende hypothese is getest om te achterhalen hoe het gebruik van ciclosporine leidt tot een lagere mycofenolzuur blootstelling: normaal gesproken zorgt het enzym MRP2 ervoor, in zowel ratten als mensen, dat afbraakproducten van mycofenolzuur via de gal in de darm terecht komen. In de darm worden de afbraakproducten teruggevormd tot mycofenolzuur, wat weer opnieuw door de darm wordt opgenomen en in het bloed terecht komt. Dit wordt de enterohepatische kringloop genoemd en komt neer op het "recyclen" van mycofenolzuur. Ciclosporine blokkeert het functioneren van MRP2. Hierdoor kunnen afbraakproducten van mycofenolzuur niet meer via de gal naar de darm worden afgevoerd, en kan er dus ook geen recycling meer plaatsvinden. Het resultaat is dat de concentratie van mycofenolzuur in het bloed lager is, dan wanneer er wel recycling zou zijn.

De hypothese kon in het rattenexperiment bevestigd worden. Het bleek namelijk dat de blootstelling aan mycofenolzuur in het bloed van de genetisch gemodificeerde ratten die de combinatie MMF en ciclosporine kregen niet anders was dan de blootstelling aan mycofenolzuur in genetisch gemodificeerde ratten die geen ciclosporine kregen. Het verschil in mycofenolzuur blootstelling dat er wel is in aanwezigheid van MRP2, valt dus weg in afwezigheid van MRP2.

3.2.2 De oorzaak van de invloed van de transplantaat functie en de albumine concentratie

In hoofdstuk 2.6 is onderzocht hoe het komt dat een slecht functionerend niertransplantaat en een lage albumine concentratie leiden tot een lage blootstelling aan mycofenolzuur in het bloed. De volgende hypothese is getest: mycofenolzuur is in het bloed sterk gebonden aan albumine: mycofenolzuur is voor

ongeveer 97-98% gebonden aan albumine en is voor de resterende 2-3% ongebonden in het bloed aanwezig. Wanneer de albumine concentratie in het bloed laag is, neemt de binding van mycofenolzuur aan albumine af. Ook wanneer het niertransplantaat slecht functioneert, kan dat indirect leiden tot een lagere binding van mycofenolzuur aan albumine. Als de binding aan albumine afneemt, zal er meer ongebonden mycofenolzuur in het bloed aanwezig zijn. Voor mycofenolzuur wordt aangenomen dat alleen de ongebonden fractie beschikbaar is om uit het lichaam verwijderd te worden. Bij mycofenolzuur gebeurt dat in de lever door middel van omzetting van mycofenolzuur in afbraakproducten. Bij een lage albumine concentratie of bij een slecht functionerend niertransplantaat zal er dus door een lagere binding van mycofenolzuur aan albumine, meer ongebonden mycofenolzuur in het bloed aanwezig zijn. Hierdoor zal het aanbod ongebonden mycofenolzuur in de lever toenemen. Een hoger aanbod zal ertoe leiden dat er per tijdseenheid meer mycofenolzuur wordt omgezet in afbraakproducten. Het resultaat is dat de totale blootstelling aan mycofenolzuur (ongebonden + gebonden aan albumine) in het bloed zal dalen.

Om deze hypothese te testen is een mechanistisch computermodel ontwikkeld, waarin het proces van de binding van mycofenolzuur aan albumine mathematisch wordt beschreven. Het model is ontwikkeld met behulp van gegevens van 88 niertransplantaties patiënten over de blootstelling aan totaal mycofenolzuur, de blootstelling aan niet-gebonden mycofenolzuur en de blootsteling aan afbraakproducten van mycofenolzuur. Het model kon de hypothese bevestigen. Het bleek dat lage albumine concentraties in het bloed, slecht functioneren van het transplantaat en hoge concentraties van het afbraakproduct van mycofenolzuur, de ongebonden fractie van mycofenolzuur doen stijgen, terwijl de totale mycofenolzuur concentratie afneemt. De observatie dat hoge concentraties van het afbraakproduct van mycofenolzuur ook leiden tot een toename van de ongebonden fractie, duidt erop dat het afbraakproduct ook aan albumine bindt en mycofenolzuur uit diens albuminebinding kan verdringen.

4 HYPOTHESE 2: IDEALE MYCOFENOLZUUR BLOOTSTELLING KAN IN MEER PATIËNTEN BEREIKT WORDEN DOOR THERAPEUTIC DRUG MONITORING

De tweede hypothese was dat de ideale blootstelling aan mycofenolzuur in het bloed in meer patiënten bereikt kan worden door therapeutic drug monitoring en is onderzocht in de hoofdstukken 3.1 en 3.2. Therapeutic drug monitoring is het periodiek meten van de mycofenolzuur blootstelling, waarna de dosering MMF verhoogd wordt als in een patiënt een te lage mycofenolzuur blootstelling gemeten is, of waarna de dosering verlaagd wordt als in een patiënt een te hoge blootstelling gemeten is.

4.1 Voorspelling van het nut van therapeutic drug monitoring

Hoofdstuk 3.1 beschrijft een computer simulatie studie, waarin is onderzocht of en hoe snel de blootstelling aan mycofenolzuur met behulp van therapeutic drug monitoring binnen de ideale grenzen van 30 – 60 mg*h/L gebracht zou kunnen worden. Voor de simulatie zijn gegevens van 45 niertransplantatie patiënten gebruikt over blootstelling aan mycofenolzuur. Deze gegevens vormden de basis voor berekeningen van de blootstelling aan mycofenolzuur in het bloed wanneer deze patiënten een andere dosis MMF zouden hebben gekregen dan dat ze in werkelijkheid gehad hadden. Hierbij

is een hogere dosis nagebootst als in de patiënten een te lage blootstelling gemeten was en is een lagere dosis nagebootst bij een te hoge blootstelling. Dit gesimuleerde doseringsschema op basis van therapeutic drug monitoring is vergeleken met een gesimuleerd doseringsschema waarbij de standaard dosis van twee maal daags 1 gram is nagebootst. De resultaten lieten zien dat bij een doseringsschema op basis van therapeutic drug monitoring 76% van de patiënten een mycofenolzuur blootstelling heeft tussen de 30 – 60 mg*h/L al op dag 7 na transplantatie, ten opzichte van 13% bij het standaard doseringsschema. Om dit te bereiken, moest er bij het doseringsschema op basis van therapeutic drug monitoring een twee maal zo hoge MMF dosering (twee maal daags 2 gram) worden nagebootst in meer dan de helft van de patiënten. Op basis van dit onderzoek kan voorspeld worden dat met behulp van therapeutic drug monitoring meer niertransplantatie patiënten sneller een adequate blootstelling aan mycofenolzuur zullen hebben dan wanneer het standaard MMF doseringsschema toegepast wordt.

4.2 Variabiliteit in de blootstelling aan mycofenolzuur binnen een patiënt in de tijd

In hoofdstuk 3.2 is bekeken hoe groot de variabiliteit is in de blootstelling aan mycofenolzuur in het bloed binnen een en dezelfde patiënt in de tijd. De mycofenolzuur blootstelling binnen een patiënt in de tijd is variabel wanneer bijvoorbeeld de blootstelling op het ene moment in een patiënt 20 mg*h/L is, terwijl enkele dagen later de blootstelling in diezelfde patiënt 46 mg*h/L is bij ongewijzigde MMF dosering. Een dergelijk grote variabiliteit binnen een patiënt in de tijd maakt dat een meting van de mycofenolzuur blootstelling op een bepaald moment weinig zegt over de blootstelling enkele dagen later. Het is dan nauwelijks uitvoerbaar om de blootstelling aan mycofenolzuur in een patiënt binnen de ideale grenzen van 30 - 60 mg*h/L te krijgen én te houden.

De blootstelling aan mycofenolzuur in het bloed is voor 45 niertransplantatie patiënten gevolgd op 9 achtereenvolgende tijdstippen gedurende de eerste vijf maanden na transplantatie. Binnen de 9 tijdstippen liggen 8 intervallen waarin variaties in de blootstelling aan mycofenolzuur binnen een patiënt in de tijd bestudeerd zijn. Per interval konden maximaal 0 tot 3 punten gescoord worden. Een score van 0 of 1 is toegekend wanneer er kleine schommelingen in de mycofenolzuur blootstelling binnen een patiënt in de tijd waren, en een score van 2 of 3 is toegekend wanneer er grote schommelingen waren. Het onderscheid tussen kleine en grote schommelingen in de tijd is belangrijk vanwege het feit dat kleine variaties voor een arts niet interessant zijn, omdat het geen reden is voor dosisaanpassing, terwijl grote variaties wel een indicatie zijn om de dosis aan te passen. De maximale totale score bedroeg het aantal intervallen (8) vermenigvuldigd met de maximale score per interval (3) is 24.

De gemiddelde totale score voor de variabiliteit in de blootstelling aan mycofenolzuur in het bloed binnen een en dezelfde patiënt in de tijd bleek klein te zijn: 3.4. Op basis van deze lage score kan geconcludeerd worden dat voor effectieve therapeutic drug monitoring van mycofenolzuur niet onpraktisch veel metingen per patiënt nodig zullen zijn.

5 MMF IN DE HEMATOLOGIE

Gezien het succes van MMF binnen de niertransplantatie geneeskunde, wordt dit geneesmiddel ook in toenemende mate toegepast binnen andere specialismen waarbij onderdrukking van het afweersysteem nodig is. Een van deze specialismen is de hematologie, waar patiënten met verschillende vormen van leukemie een transplantatie ondergaan van stamcellen van het afweersysteem. Hierbij wordt het aan kanker lijdende afweersysteem van de patiënt eerst helemaal vernietigd met chemotherapie, waarna stamcellen van het gezonde afweersysteem van de donor worden geïntroduceerd. Omdat dit nieuwe

afweersysteem het lichaam van de patiënt als "vreemd" kan herkennen, zou een afstotingsreactie kunnen ontstaan gericht tegen het lichaam van de patiënt, en dan met name de huid, darmen en lever. Een dergelijke reactie wordt graft-versus-host disease genoemd. Om graft-versus-host disease te voorkomen worden geneesmiddelen ingezet die het afweersysteem onderdrukken.

Sinds kort wordt ook MMF ingezet om graft-versus-host disease te voorkomen. Omdat echter MMF niet ontwikkeld is voor dit doeleinde heeft de fabrikant nooit onderzocht welke dosis van het geneesmiddel het beste effect oplevert. Om die reden maken hematologen met name gebruik van ervaringen uit de niertransplantatie, waardoor er voor stamcel transplantatie patiënten gekozen is voor dezelfde standaard MMF dosering als voor niertransplantatie patiënten: 15 mg/kg lichaamsgewicht, wat bij de meeste patiënten neerkomt op een dosering van twee maal daags 1 gram. In hoofdstuk 4.1 is bij 15 stamcel transplantatie patiënten in de eerste maand na transplantatie onderzocht welke blootstelling aan mycofenolzuur in het bloed ontstaat na een dosering van twee maal daags 15 mg/kg lichaamsgewicht MMF. De resultaten lieten zien dat de gemiddelde blootstelling mycofenolzuur in het bloed opmerkelijk laag is na toediening van de genoemde standaarddosering: 18 mg*h/L. Dit is grofweg twee maal zo laag als de gemiddelde blootstelling die normaal gesproken in niertransplantatie patiënten gevonden wordt. Binnen de niertransplantatie zou deze lage blootstelling aan mycofenolzuur worden beschouwd als onvoldoende om het afweersysteem goed te onderdrukken. Er kan geconcludeerd worden dat MMF waarschijnlijk hoger of vaker dan twee maal daags 1 gram gedoseerd moet worden bij stamcel transplantatie patiënten om het afweersysteem voldoende te onderdrukken.

6 CONCLUSIES

In hoofdstuk 5 worden alle resultaten uit de voorgaande hoofdstukken bediscussieerd en wordt er antwoord gegeven op de vraag of de twee hypotheses uit hoofdstuk 1.2 bevestigd zijn of verworpen moeten worden. De hypotheses waren:

1. dat ideale blootstelling aan mycofenolzuur in het bloed in meer patiënten bereikt kan worden door de MMF dosering toe te spitsen op individuele patiëntkenmerken.
2. dat de ideale blootstelling aan mycofenolzuur in het bloed in meer patiënten bereikt kan worden door middel van therapeutic drug monitoring.

Geconcludeerd wordt dat voor beide hypotheses bewijs gevonden is, waardoor de hypotheses bevestigd kunnen worden.

Tot slot wordt op basis van alle resultaten van het onderzoek, beschreven in hoofdstukken 2.1 tot en met 3.2, een aanbeveling gegeven over welke MMF dosering op welk moment na transplantatie het beste past bij een individuele patiënt om de ideale blootstelling aan mycofenolzuur in het bloed te bereiken. De aanbeveling bestaat uit:

1. een startdosering van MMF van twee maal daags 1.5 gram wanneer de patiënt gelijktijdig ook ciclosporine gebruikt, in plaats van de gebruikelijke 2 maal daags 1 gram, om de blootstelling aan mycofenolzuur in het bloed vanaf het begin boven de 30 mg*h/L te krijgen (hoofdstuk 2.2 en 3.1)
2. een meting van de mycofenolzuur blootstelling in de eerste week na transplantatie met zonodig daaropvolgende dosisaanpassing, om ook de blootstelling in de patiënten die niet binnen de ideale grenzen van 30 - 60 mg*h/L zaten daar alsnog te krijgen (hoofdstuk 3.1 en 3.2)

3. een meting van de mycofenolzuur blootstelling met zonodig daaropvolgende dosisaanpassing na een maand, als de nierfunctie en de albumine concentratie, en daardoor de mycofenolzuur blootstelling, gestabiliseerd zijn, om de blootstelling binnen de ideale grenzen van 30 - 60 mg*h/L te houden (hoofdstuk 2.2, 2.4 en 3.2).
4. Aanvullende metingen van de mycofenolzuur blootstelling met daaropvolgende dosisaanpassingen kunnen worden gedaan wanneer de ciclosporine dosering wordt verlaagd, of wanneer er een plotselinge verandering optreedt in het functioneren van het transplantaat of in de albumine concentratie (hoofdstuk 2.1, 2.4, 2.6 en 3.2).

Al met al kan geconcludeerd worden dat dit onderzoek belangrijke aanvullende informatie heeft opgeleverd om de toepassing van MMF te verbeteren. De hiervoor gebruikte methodiek is ook toepasbaar op andere geneesmiddelen en verdient navolging. Als een nieuw geneesmiddel op de markt komt, is er slechts beperkte gebruikservaring. Dit proefschrift laat zien hoe belangrijk het is om grootschalige gebruikservaring te analyseren om de toepassing van het geneesmiddel te optimaliseren.

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OVER DE AUTEUR

Reinier M. van Hest werd geboren op 6 september 1977 te Leiden. In 1996 behaalde hij het VWO diploma aan het Carolus Borromeus College te Helmond, waarna hij aanving met de studie Farmacie aan de Universiteit Utrecht. Tijdens de studie voerde hij een bioanalytisch onderzoeksproject uit gericht op de analyse van het proteoom van penicilline producerende bacteriën bij DSM te Delft. Daarnaast verrichtte hij een extracurriculair onderzoeksproject over de geschiktheid van cystatine C als marker voor de nierfunctie bij kinderen binnen de afdelingen Apotheek en Klinische Chemie van het toenmalige Academisch Ziekenhuis Rotterdam (nu Erasmus MC). In 2000 legde hij cum laude het doctoraal examen af van de studie Farmacie. In 2003 volbracht hij het apothekersexamen. In maart 2003 begon hij in de Apotheek van het Erasmus MC te Rotterdam het ZAPIKO-traject (opleiding tot ziekenhuisapotheker gecombineerd met promotie-onderzoek). Het onderzoeksdeel van dit traject stond onder supervisie van Prof. dr. A.G. Vulto, dr. R.A.A. Mathôt en dr. T. van Gelder en heeft geresulteerd in het voorliggende proefschrift. Voor een onderdeel van het onderzoek ontving hij in 2005 een Young Investigator Award van het American Transplant Congress.

Sinds januari 2005 is hij tevens plaatsvervangend onderwijscoördinator voor het klinisch geneesmiddelenonderwijs in het curriculum Erasmusarts 2007 aan de Erasmus Universiteit Rotterdam.

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